

PCT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 15 March 2001 (15.03.01)	
International application No. PCT/GB00/02546	Applicant's or agent's file reference SCB/52945001
International filing date (day/month/year) 30 June 2000 (30.06.00)	Priority date (day/month/year) 30 June 1999 (30.06.99)
Applicant ROBERTS, David, John et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
 30 January 2001 (30.01.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Juan Cruz Telephone No.: (41-22) 338.83.38
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TENT COOPERATION TREAT

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference SCB/52945001	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 00/ 02546	International filing date (day/month/year) 30/06/2000	(Earliest) Priority Date (day/month/year) 30/06/1999
Applicant ISIS INNOVATION LTD		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

TREATMENT OF DENDRITIC CELLS FOR INDUCTION OF IMMUNE TOLERANCE

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

T/GB 00/02546

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N5/08 G01N33/50 A61K39/395 A61K39/005 A61K38/17
A61K35/14 A61K39/00 A61P37/00 A61P33/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, MEDLINE, LIFESCIENCES, CHEM ABS Data, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 06848 A (BLOOD RES CENTER) 15 April 1993 (1993-04-15) the whole document	33-35, 38-41, 54-62, 75,76
X	MCCORMICK C J ET AL: "Intercellular adhesion molecule-1 and CD36 synergize to mediate adherence of Plasmodium falciparum-infected erythrocytes to cultured human microvascular endothelial cells." JOURNAL OF CLINICAL INVESTIGATION, vol. 100, no. 10, 15 November 1997 (1997-11-15), pages 2521-2529, XP000971964 the whole document	54-62, 75,76
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

11 January 2001

Date of mailing of the international search report

25/01/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Teyssier, B



INTERNATIONAL SEARCH REPORT

International Application No.

/GB 00/02546

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 05191 A (UAB RESEARCH FOUNDATION) 23 February 1995 (1995-02-23) the whole document	33,34, 38-40, 43,44, 46, 49-51, 53,75, 104-107
X	WO 96 33736 A (AFFYMAX TECH NV ;BARUCH DROR I (US); HOWARD RUSSELL J (US); PASLOS) 31 October 1996 (1996-10-31) the whole document	33,34, 36, 38-40, 42,43, 46,47, 49-51, 53,75, 104-107
X	WO 90 15609 A (MED TAL INC) 27 December 1990 (1990-12-27) the whole document	108-111
A	ALBERT M L ET AL: "Immature dendritic cells phagocytose apoptotic cells via alpha V beta 5 and CD36, and cross-present antigens to cytotoxic T lymphocytes" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 188, no. 7, 5 October 1998 (1998-10-05), pages 1359-1368, XP000906793	
P,X	URBAN B C ET AL: "Modulation of dendritic cell maturation and function." TISSUE ANTIGENS, vol. 55, no. Supplement 1, 2000, page 61 XP000971966 7th Workshop and Conference on Human Leucocyte Differentiation Antigens; Harrogate, England; 20-24 June 2000 abstract I. 19	1-115
P,X	URBAN B C ET AL: "Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells." NATURE, vol. 400, no. 6739, 1 July 1999 (1999-07-01), pages 73-77, XP002156922 the whole document	1-115



This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-53, 77-115

Methods of treating antigen presenting cells, including dendritic cells, to induce immune tolerance; the resulting antigen presenting cells preparations and their use as a medicament.

Methods of identifying agonists of CD36 or CD51; pharmaceutical compositions containing CD36 or CD51 agonists and their use as a medicament.

Methods of identifying agonists of a beta-integrin associated with CD51. Pharmaceutical composition containing a beta-integrin agonist and a CD51 agonist. Use of a beta-integrin agonist to induce tolerance in antigen presenting cells; tolerised cell composition.

Pharmaceutical composition containing a thrombospondin agonist. Use of a thrombospondin agonist to induce tolerance in antigen presenting cells; tolerised cell composition.

Pharmaceutical composition containing a negatively charged phospholipid. Use of a negatively charged phospholipid to induce tolerance in antigen presenting cells; tolerised cell composition.

2. Claims: 54-76

Methods of identifying a molecule capable of preventing the adherence of red blood cells infected with a malarial parasite to human dendritic cells; said molecule and pharmaceutical compositions comprising it.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

1/ Lack of clarity

Claims 1-3, 33, 37-39, 43, 46-50, 53, 73-76, 82-85, 87 and 115 relate to compounds, to pharmaceutical compositions comprising compounds or to methods using compounds in which said compounds are defined solely by reference to desirable characteristics or properties, namely the agonism of the cells surface receptors CD36 and/or CD51 or the prevention of the adherence of red blood cells infected with a malarial parasite to human dendritic cells. The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, disclosed and supported, namely antibodies to CD36 and/or CD51 (as applicable), Pf-EMP-1, thrombospondin, fragments and derivatives of said antibodies and proteins, and negatively charged phospholipids, as mentioned in claims 4, 6-8, 34-36, 40-42, 44, 45, 51, 52 and 88.

2/ Inconsistencies in claims 9, 35-37 and 102-103

Claim 9 relates to "a method as claimed in any one of claims 40 to 47" while said claims 40-47 relate to compounds and compositions thereof; claim 9 is therefore inconsistent. In view of the context of these claims, this International Search Authority assumed that claim 9 refers to claims 1-8.

Claims 35-36 and 37 relate to a composition as claimed in claim 20 and 19, respectively, while said claims 20 and 19 relate to a method of identifying ligands; claims 35-37 are therefore inconsistent. In view of the context of these claims, this International Search Authority assumed that claims 35-36 refer to claim 34 and that claim 37 refers to claim 33.

Claims 102-103 relate to "a preparation of cells obtainable by the the method as claimed in any one of claims 97 to 100" while claim 97 relates to a pharmaceutical composition of a beta-integrin. Claims 98-100 do relate to a method suitable to obtain a composition of claims 102-103. Consequently, the part of claims 102-103 which relate to claim 97 was disregarded.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/02546

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 115 is directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/02546

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9306848	A	15-04-1993	AU 2795892 A	03-05-1993
			AU 2797892 A	03-05-1993
			AU 2861292 A	03-05-1993
			WO 9306849 A	15-04-1993
			WO 9306850 A	15-04-1993
WO 9505191	A	23-02-1995	AU 697624 B	15-10-1998
			AU 7565294 A	14-03-1995
			CA 2169360 A	23-02-1995
			EP 0716609 A	19-06-1996
			JP 9505555 T	03-06-1997
WO 9633736	A	31-10-1996	AU 5851296 A	18-11-1996
WO 9015609	A	27-12-1990	EP 0477270 A	01-04-1992
			US 5135922 A	04-08-1992
			US 5262406 A	16-11-1993

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REC'D 10 OCT 2001

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SCB/52945/001	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB00/02546	International filing date (day/month/year) 30/06/2000	Priority date (day/month/year) 30/06/1999
International Patent Classification (IPC) or national classification and IPC A61K39/00		
Applicant ISIS INNOVATION LTD		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 9 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 15 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 30/01/2001	Date of completion of this report 04.10.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Wagner, R Telephone No. +49 89 2399 7357



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02546

I. Basis of this report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-117 as originally filed

Claims, No.:

1-82 as received on 16/08/2001 with letter of 13/08/2001

Drawings, sheets:

1/13-13/13 as originally filed

Sequence listing part of the description, pages:

2, filed with the letter of 28.8.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02546

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 27-30,33,37,39,44-48,51-68,72.

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☒ the claims, or said claims Nos. 28,29,51-60,61-68, are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 27,30,33,37,39,44,45-48,72 .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02546

- ☐ restricted the claims.
 - ☐ paid additional fees.
 - ☐ paid additional fees under protest.
 - ☐ neither restricted nor paid additional fees.
2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
- ☐ complied with.
 - ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:
- ☒ all parts.
 - ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26,31,32, 38,40-43, 49,50, 69-70, 73-82
	No:	Claims	34-36, 71
Inventive step (IS)	Yes:	Claims	1-26,31,32,34-36, 38,40-43, 49,50, 69-70, 73-82
	No:	Claims	34-36, 71
Industrial applicability (IA)	Yes:	Claims	1-26,31,32,34-36, 38,40-43, 49,50, 69-71, 73-82
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/02546

Re Item I

Basis of the report

Two sheets similar to figures 8 or 9 were filed with the letter dated 13.08.2001. The letter does not refer to the sheets and the sheets were not identified with a figure number. Therefore the filed sheets cannot be considered as being part of the basis of the report.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Reference is made to the following documents:

- D1: URBAN B C ET AL: 'Modulation of dendritic cell maturation and function.' TISSUE ANTIGENS, vol. 55, no. Supplement 1, 2000, page 61 XP000971966 7th Workshop and Conference on Human Leucocyte Differentiation Antigens; Harrogate, England; 20-24 June 2000
- D2: URBAN B C ET AL: 'Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells.' NATURE, vol. 400, no. 6739, 1 July 1999 (1999-07-01), pages 73-77, XP002156922
- D3: WO 95 05191 A (UAB RESEARCH FOUNDATION) 23 February 1995 (1995-02-23)
- D4: WO 93 06848 A (BLOOD RES CENTER) 15 April 1993 (1993-04-15)
- D5: WO9636349
- D6: WO 96 33736 A (AFFYMAX TECH NV ;BARUCH DROR I (US); HOWARD RUSSELL J (US); PASLOS) 31 October 1996 (1996-10-31)
- D7: WO 90 15609 A (MED TAL INC) 27 December 1990 (1990-12-27)
- D8: Experimental Parasitology 89, 78-85(1998)
- D9: MCCORMICK C J ET AL: 'Intercellular adhesion molecule-1 and CD36 synergize to mediate adherence of Plasmodium falciparum-infected erythrocytes to cultured human microvascular endothelial cells.' JOURNAL OF CLINICAL INVESTIGATION, vol. 100, no. 10, 15 November 1997 (1997-11-15), pages 2521-2529, XP000971964
- D10: ALBERT M L ET AL: 'Immature dendritic cells phagocytose apoptotic cells via

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/02546

alpha V beta 5 and CD36, and cross-present antigens to cytotoxic T lymphocytes'
JOURNAL OF EXPERIMENTAL MEDICINE, vol. 188, no. 7, 5 October 1998
(1998-10-05), pages 1359-1368, XP000906793

The documents D5 and D8 were not cited in the international search report and are introduced by the examiner (Guidelines, VI-7.24).

1. No international search report has been established for the subject-matter of claims 27, 30, 33, 37, 39, 44, 45-48, 72 these claims can therefore not be subject of the present international preliminary , examination (Rule 66.1(e) PCT).
2. Claims 28, 29, 34, 61-68 are not sufficiently disclosed (Article 5 PCT) and not supported by the description (Article 6 PCT). The present application does not comprise any substantive support for the hypothesis that a pharmaceutical composition comprising: a) an agonist to the CD36, CD51 or thrombospondin receptor or b) apoptotic cells or negatively charged phospholipids does induce peripheral immune tolerance in vivo when administered directly as a pharmaceutical composition to a subject but shows only that in vitro the maturation of isolated immature dendritic cells can be inhibited by these agonists and that the dendritic cells which have been prevented from maturation have a reduced inflammatory effect in vivo in comparison to controls.
3. Claims 51-60 are not sufficiently disclosed (Article 5 PCT) and not supported by the description (Article 6 PCT) because the present application does not provide any substantive support that the β -integrins associated to the CD51 play any role in the inhibition of the maturation of immature dendritic cells.

Re Item IV

Lack of unity of invention

The present set of claims contains two inventions which are not linked by an inventive common concept (Rule 13.1 PCT). (see also International Search Report -Box II).

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/02546

The subject-matter of claims 1-72 relates to methods of treating antigen presenting cells (APC) with agonists to CD36 and CD51 in order to induce immune tolerance in said cells. The medical use of pretreated APCs. Pharmaceutical compositions comprising CD36/CD51 agonists or thrombospondin or negatively charged phospholipids. Methods of identifying agonists of CD36/51, agonists of beta-integrin associated with CD51.

Claims 73-82 are directed to methods of identifying a molecule capable of preventing the adherence red blood cells infected with a malarial parasite

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement.

1. Documents D1 and D2 were published after the priority date but before the international filing date of the present application. The priority document filed on 30.06.1999 contains the subject-matter disclosed in D1 and D2. Therefore said documents are not part of the prior art.
2. Claim 1 is directed to a method for inducing immune tolerance of immature mammalian dendritic cells in vitro by exposing the cells to certain agonists of the cell surface receptors CD36 and/or CD51 which are: an antibody with an affinity for an epitope of CD36 or CD51, pf-EMP-1, thrombospondin, apoptotic cells, negatively charged phospholipids. Claim 1 and dependent claims 2-7 are novel (Article 33(2) PCT) and involve an inventive step (Article 33(3) PCT) because the available prior art does not disclose a method for inducing immune tolerance in immature dendritic cells by using the above agonists nor does it give any indication that the CD36 and CD51 receptors are involved in the maturation of immature dendritic cells.
3. Claim 10 is new (Article 33(2) PCT) and involves an inventive step (Article 33(3) PCT). Because the available prior art does not disclose or indicate any relationship between the maturation of immature dendritic cells and the cell surface receptors CD36 or CD51. The methods of the dependent claims 11-26

are also new and inventive.

4. Claims 31, 32, 34-36, 38 are directed to the medical use of several ligands of the receptors CD36 or CD51 for inducing a state of immune tolerance in mammal or human. The medical use of thrombospondin is disclosed in D3 (see claim 9), the medical use of anti-CD36 antibodies is disclosed in D4 (page 7, lines 9-11) and D5 (page 8, lines 15-16 and page 2, lines 24-36), the medical use of pf-EMP-1 is disclosed in D6 (claim 33) and the medical use of phosphatidylserine is disclosed in D7 (page 4, first paragraph). Therefore claims 34-36 which are directed to the first medical use of the agonists are not new (Article 33(2) PCT). None of the prior art documents discloses or indicates that the ligands could induce a state of immune tolerance. Notwithstanding the objection regarding insufficiency of disclosure raised in item VIII, section 1 the subject-matter of claims 31, 32, 38 can be considered as new (Article 33(2) PCT) and as involving an inventive step (Article 33(3) PCT) because they are formulated as second medical use claims and directed at the induction of a state of immune tolerance..
5. Claims 40-43 directed to a method for identifying a molecule which is an agonist to the CD36 and/or CD51 receptor and/or thrombospondin receptor by detecting the effect of the agonist on the immune response. As the prior art does not disclose or indicate any relation of the CD36 or CD51 or thrombospondin receptors with a reduction of the immune response the method of claims 40-43 is new (Article 33(2) PCT) and inventive (Article 33(3) PCT).
6. Claims 8, 9, 49 and 50 are new (Article 33(2) PCT) and inventive (Article 33(3) PCT) because the prior art does not disclose or indicate the medical use of dendritic cells or APCs which have been exposed to agonists of CD36, CD51 or to the thrombospondin receptor. D10 (page 1364, paragraph 1) discloses immature dendritic cells which are exposed to anti-CD36 monoclonal antibodies but does not disclose or indicate any medical application of said immature dendritic cells.
7. The method (claims 69-70) of inducing a state of immune tolerance in APCs by exposing said cells to an agonist of CD36, CD51 or of the thrombospondin receptor is new (Article 33(2) PCT) and involves an inventive step (Article 33(3) PCT) because the prior art does not disclose or indicate any relation of the CD36

or CD51 or thrombospondin receptors with a reduction of the immune response which can be generated by APCs.

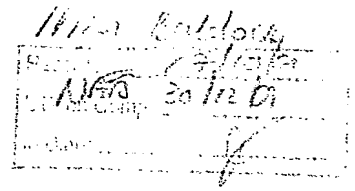
8. Claim 71 is directed to antigen-presenting cells obtainable by exposing the cells to compositions or preparations containing agonists of thrombospondin receptors or apoptotic cells. D10 discloses that immature dendritic cells are exposed to apoptotic cells (figure 3). Therefore claim 71 is not new (Article 33(2) PCT).
9. Although the role of the pf-EMP-1 plasmodium falciparum protein in the adhesion to CD36 is known and the presence of CD36 receptors on dendritic cells is also known, the method (claims 73-82) of identifying molecules capable of preventing the adherence of infected red blood cells to dendritic cells using the effect of inhibition of maturation of immature dendritic cells is new (Article 33(2) PCT) and involves an inventive step (Article 33(3) PCT). The prior art does not give any indication that the CD36 and CD51 receptors are involved in the maturation of immature dendritic cells.

Item VIII

Certain observations on the international application

1. Claims 31, 32, 34-36, 38 are not sufficiently disclosed (Article 5 PCT) and not supported by the description (Article 6 PCT). The present application does not comprise any substantive support for the hypothesis that a pharmaceutical composition comprising: a) an agonist to the CD36, CD51 or thrombospondin receptor or b) apoptotic cells or negatively charged phospholipids does induce peripheral immune tolerance in vivo when administered directly as a pharmaceutical composition to a subject but shows only that in vitro the maturation of isolated immature dendritic cells can be inhibited by these agonists and that the dendritic cells which have been prevented from maturation have a reduced inflammatory effect in vivo in comparison to controls.
2. Claim 69 is not clear (Article 6 PCT) because it refers to compositions or preparations but claims 65 to 68 are use claims.

PATENT COOPERATION TREATY



From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Rule 71.1)

To:

BOULT WADE TENNANT
Verulam Gardens
70 Gray's Inn Road
London WC1X 8BT
GRANDE BRETAGNE

Date of mailing
(day/month/year) 04.10.2001

Applicant's or agent's file reference
SCB/52945/001

IMPORTANT NOTIFICATION

International application No.
PCT/GB00/02546

International filing date (day/month/year)
30/06/2000

Priority date (day/month/year)
30/06/1999

Applicant
ISIS INNOVATION LTD

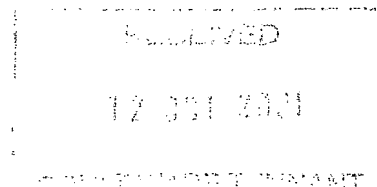
1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.



Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Digiusto, M

Tel. +49 89 2399-8162



INTERNATIONAL SEARCH REPORT

International Application No

P GB 00/02546

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N5/08 G01N33/50 A61K39/395 A61K39/005 A61K38/17
 A61K35/14 A61K39/00 A61P37/00 A61P33/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, MEDLINE, LIFESCIENCES, CHEM ABS Data, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 06848 A (BLOOD RES CENTER) 15 April 1993 (1993-04-15) the whole document	33-35, 38-41, 54-62, 75,76
X	MCCORMICK C J ET AL: "Intercellular adhesion molecule-1 and CD36 synergize to mediate adherence of Plasmodium falciparum-infected erythrocytes to cultured human microvascular endothelial cells." JOURNAL OF CLINICAL INVESTIGATION, vol. 100, no. 10, 15 November 1997 (1997-11-15), pages 2521-2529, XP000971964 the whole document	54-62, 75,76



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

11 January 2001

Date of mailing of the international search report

25/01/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Teyssier, B

INTERNATIONAL SEARCH REPORT

International Application No

P 00/02546

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 05191 A (UAB RESEARCH FOUNDATION) 23 February 1995 (1995-02-23) the whole document ---	33, 34, 38-40, 43, 44, 46, 49-51, 53, 75, 104-107
X	WO 96 33736 A (AFFYMAX TECH NV ; BARUCH DROR I (US); HOWARD RUSSELL J (US); PASLOS) 31 October 1996 (1996-10-31) the whole document ---	33, 34, 36, 38-40, 42, 43, 46, 47, 49-51, 53, 75, 104-107
X	WO 90 15609 A (MED TAL INC) 27 December 1990 (1990-12-27) the whole document ---	108-111
A	ALBERT M L ET AL: "Immature dendritic cells phagocytose apoptotic cells via alpha V beta 5 and CD36, and cross-present antigens to cytotoxic T lymphocytes" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 188, no. 7, 5 October 1998 (1998-10-05), pages 1359-1368, XP000906793 ---	
P, X	URBAN B C ET AL: "Modulation of dendritic cell maturation and function." TISSUE ANTIGENS, vol. 55, no. Supplement 1, 2000, page 61 XP000971966 7th Workshop and Conference on Human Leucocyte Differentiation Antigens; Harrogate, England; 20-24 June 2000 abstract I. 19 ---	1-115
P, X	URBAN B C ET AL: "Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells." NATURE, vol. 400, no. 6739, 1 July 1999 (1999-07-01), pages 73-77, XP002156922 the whole document -----	1-115

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

P 92/0002546

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9306848	A	15-04-1993	AU	2795892 A	03-05-1993
			AU	2797892 A	03-05-1993
			AU	2861292 A	03-05-1993
			WO	9306849 A	15-04-1993
			WO	9306850 A	15-04-1993
WO 9505191	A	23-02-1995	AU	697624 B	15-10-1998
			AU	7565294 A	14-03-1995
			CA	2169360 A	23-02-1995
			EP	0716609 A	19-06-1996
			JP	9505555 T	03-06-1997
WO 9633736	A	31-10-1996	AU	5851296 A	18-11-1996
WO 9015609	A	27-12-1990	EP	0477270 A	01-04-1992
			US	5135922 A	04-08-1992
			US	5262406 A	16-11-1993

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

To:

BOULT WADE TENNANT
Verulam Gardens
70 Gray's Inn Road
London WC1X 8BT
UNITED KINGDOM

Miss Boldyrb.
4-26/1/01
o/L 2513101
an

Date of mailing
(day/month/year)

25/01/2001

Applicant's or agent's file reference

SCB/52945001

FOR FURTHER ACTION

See paragraphs 1 and 4 below

International application No.

PCT/GB 00/ 02546

International filing date
(day/month/year)

30/06/2000

Applicant

ISIS INNOVATION LTD

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

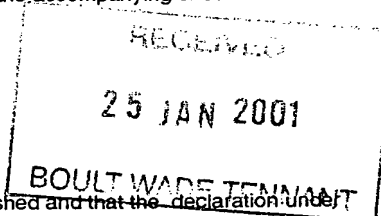
Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.



2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority

 European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Catherine Humbert



NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.



TES TO FORM PCT/ISA/220 (contin)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.



PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

BOULT WADE TENNANT
Verulam Gardens
70 Gray's Inn Road
London WC1X 8BT
GRANDE BRETAGNE

Miss. Baldeck
acc. 13/7/01
OL: 13/8/01
acc.

by fax and post

PCT

WRITTEN OPINION

(PCT Rule 66)

FAX: 44 20 7430 7600

Date of mailing
(day/month/year)

13.07.2001

Applicant's or agent's file reference
SCB/52945/001

REPLY DUE

within 1 month(s)
from the above date of mailing

International application No.
PCT/GB00/02546

International filing date (day/month/year)
30/06/2000

Priority date (day/month/year)
30/06/1999

International Patent Classification (IPC) or both national classification and IPC
A61K39/00

Applicant

ISIS INNOVATION LTD

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.
2. This opinion contains indications relating to the following items:
 - I ☒ Basis of the opinion
 - II ☐ Priority
 - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☒ Lack of unity of invention
 - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain document cited
 - VII ☐ Certain defects in the international application
 - VIII ☒ Certain observations on the international application
3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.
4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 30/10/2001.

Name and mailing address of the International
preliminary examining authority:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4165

Authorized officer / Examiner

Wagner, R

Formalities officer (incl. extension of time limits)

Digiusto, M

Telephone No. +49 39 2399 8162



WRITTEN OPINION

International application No. PCT/GB00/02546

1. Basis of the opinion

1. With regard to the elements of the international application (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"):

Description, pages:

1-117 as originally filed

Claims, No.:

1-115 as originally filed

Drawings, sheets:

1/13-13/13 as originally filed

Sequence listing part of the description, pages:

2, filed with the letter of 28.08.2000

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

- ☐ the entire international application,
☒ claims Nos. 1-3, 13, 14, 33-39, 43-50, 53, 73-115,

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 81, 86, 89, 93, 94 are so unclear that no meaningful opinion could be formed (*specify*):
see separate sheet
- ☒ the claims, or said claims Nos. 13, 14, 34-36, 44, 45, 77-81, 86-103, 92-103, 104-111, 112-114 are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 1-3, 33, 37-39, 43, 46-50, 53, 73-76, 82-85, 87, 115.

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

WRITTEN OPINIONInternational application No. **PCT/GB00/02546**

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.
- 2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:
see separate sheet
- 3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:
 - ☒ all parts.
 - ☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or Industrial applicability; citations and explanations supporting such statement

- 1. Statement
 - Novelty (N) Claims 40-42, 51, 52
 - Inventive step (IS) Claims 54-62
 - Industrial applicability (IA) Claims
- 2. Citations and explanations
see separate sheet

VIII. Certain observations on the International application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/GB00/02546

Re Item III**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

Reference is made to the following documents:

- D1: URBAN B C ET AL: 'Modulation of dendritic cell maturation and function.' TISSUE ANTIGENS, vol. 55, no. Supplement 1, 2000, page 61 XP000971966 7th Workshop and Conference on Human Leucocyte Differentiation Antigens; Harrogate, England; 20-24 June 2000
- D2: URBAN B C ET AL: 'Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells.' NATURE, vol. 400, no. 6739, 1 July 1999 (1999-07-01), pages 73-77, XP002156922
- D3: WO 95 05191 A (UAB RESEARCH FOUNDATION) 23 February 1995 (1995-02-23)
- D4: WO 93 06848 A (BLOOD RES CENTER) 15 April 1993 (1993-04-15)
- D5: WO9636349
- D6: WO 96 33736 A (AFFYMAX TECH NV ;BARUCH DROR I (US); HOWARD RUSSELL J (US); PASLOS) 31 October 1996 (1996-10-31)
- D7: WO 90 15609 A (MED TAL INC) 27 December 1990 (1990-12-27)
- D8: Experimental Parasitology 89, 78-85(1998)
- D9: MCCORMICK C J ET AL: 'Intercellular adhesion molecule-1 and CD36 synergize to mediate adherence of Plasmodium falciparum-infected erythrocytes to cultured human microvascular endothelial cells.' JOURNAL OF CLINICAL INVESTIGATION, vol. 100, no. 10, 15 November 1997 (1997-11-15), pages 2521-2529, XP000971964
- D10: ALBERT M L ET AL: 'Immature dendritic cells phagocytose apoptotic cells via alpha V beta 5 and CD36, and cross-present antigens to cytotoxic T lymphocytes' JOURNAL OF EXPERIMENTAL MEDICINE, vol. 188, no. 7, 5 October 1998 (1998-10-05), pages 1359-1368, XP000906793

The documents D5 and D8 were not cited in the international search report and are introduced by the examiner (Guidelines, VI-7.24). Copies of the documents are appended hereto.

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/GB00/02546

1. No international search report has been established for claims 1-3,33,37-39,43,46-50,53,73-76,82-85,87,115. These claims are not subject of the present international preliminary examination (Rule 66.1(e) PCT).
2. Claims 13 and 14 are not sufficiently disclosed (Article 5 PCT) and not supported by the description (Article 6 PCT). The present application does not comprise any substantive support for the hypothesis that a dendritic cell preparation has an immunosuppressant therapeutic effect (see also hypothetical wording in example 13).
3. Claims 34-36, 44-45, 104-111 are not sufficiently disclosed (Article 5 PCT) and not supported by the description (Article 6 PCT). The present application does not comprise any substantive support for the hypothesis that a pharmaceutical composition comprising: a) an agonist to the CD36, CD51 or thrombospondin receptor or b) apoptotic cells or negatively charged phospholipids does induce peripheral immune tolerance when administered to a subject but shows only that in vitro the maturation of isolated immature dendritic cells can be inhibited by these agonists.
4. Claims 81, 86, 89, 93, 94 are not clear (Article 6 PCT) because they are formulated to include the features of other independent claims. As the latter independent claims also include by definition the features of the claims which they are dependent on the scope of claims 81, 86, 89, 93, 94 becomes unclear.
5. Claims 77-81 and 86-103, 112-114 are not sufficiently disclosed over the whole breadth of their scope (Article 5 PCT) and not supported by the description (Article 6 PCT). These claims refer to antigen presenting cells in general which include immature and mature macrophages, B-lymphocytes or monocytes. The inhibition of the maturation of immature dendritic cells cannot be extrapolated without substantive support to all antigen presenting cells because immature dendritic cells are known to have specialised functions as for example phagocytosis of apoptic cell and their cross presentation to cytotoxic T lymphocytes. This function is not present in mature dendritic cells and in macrophages (see D10, abstract).

6. Claims 92-103 are not sufficiently disclosed (Article 5 PCT) and not supported by the description (Article 6 PCT) because the present application does not provide any substantive support that the β -integrins associated to the CD51 play any role in the inhibition of the maturation of immature dendritic cells.

Re Item IV

Lack of unity of invention

The present set of claims contains two inventions which are not linked by an inventive common concept (Rule 13.1 PCT). (see also International Search Report -Box II).

The subject-matter of claims 1-53 and 77-115 relates to methods of treating antigen presenting cells (APC) with agonists to CD36 and CD51 in order to induce immune tolerance in said cells. The medical use of pretreated APCs. Pharmaceutical compositions comprising CD36/CD51 agonists or thrombospondin or negatively charged phospholipids. Methods of identifying agonists of CD36/51, agonists of beta-integrin associated with CD51.

Claims 54-76 are directed to methods of identifying a molecule capable of preventing the adherence red blood cells infected with a malarial parasite

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Documents D1 and D2 were published after the priority date but before the international filing date of the present application. As the validity of the claimed priority could not be checked in the present preliminary examination, D1 and D2 are considered not to be part of the prior art.
2. The first claim to be examined (see item III) is claim 4 which is dependent on claims 1 or 2 and which includes the features of said claims. Claim 4 is considered to be directed to a method for inducing immune tolerance of immature mammalian dendritic cells in vitro by exposing the cells to agonists of the cell surface



**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/GB00/02546

receptors CD36 and/or CD51 which are: an antibody with an affinity for an epitope of CD36 ... (see claim 4)... phospholipid. Notwithstanding the objections raised in item VIII the subject-matter of claim 4 and of dependent claims 5-12 is novel (Article 33(2) PCT) and involves an inventive step (Article 33(3) PCT) because the available prior art does not disclose a method for inducing immune tolerance in immature dendritic cells nor does it give any indication that the CD36 and CD51 receptors are involved in the maturation of immature dendritic cells.

3. Claim 15 being completed by the missing feature of claim 26 (see item VIII, section 6) is new (Article 33(2) PCT) and involves an inventive step (Article 33(3) PCT). Because the available prior art does not disclose or indicate any relation between the maturation of immature dendritic cells and the cell surface receptors CD36 or CD51. The methods of the dependent claims 16-25 and 27 to 32 are also new and inventive.
4. Claims 40-42 and 51, 52 are directed to the medical use of several ligands of the receptors CD36 or CD51. The medical use of thrombospondin is disclosed in D3 (see claim 9), the medical use of anti-CD36 antibodies is disclosed in D4 (page 7, lines 9-11) and D5 (page 8, lines 15-16 and page 2, lines 24-36), the medical use of pf-EMP-1 is disclosed in D6 (claim 33) and the medical use of phosphatidylserine is disclosed in D7 (page 4, first paragraph). Therefore the subject-matter of claims 40-42 and 51, 52 is not new (Article 33(2) PCT).
5. The methods for identifying a molecule capable of preventing the adherence of infected red blood cells to human dendritic cells of claims 54 and 55 and of the respective dependent claims 56-62 are new (Article 33(2) PCT). D8 and D9 (abstracts) disclose that CD36 and thrombospondin are involved in the adhesion of Plasmodium falciparum infected cell to human cells. It is therefore obvious for the skilled person that a binding or adhesion assay between the infected erythrocytes and CD36 or thrombospondin can be used to screen for molecules preventing the adhesion. Therefore the subject-matter of claims 54 and 55 does not involve an inventive step (Article 33(3) PCT). The features of dependent claims 56 to 62 do not appear to confer an inventive step to the method.
6. Although the role of the pf-EMP-1 plasmodim falciparum protein in the adhesion to

CD36 is known and the presence of CD36 receptors on dendritic cells is also known, the methods (claims 63-72) of identifying molecules capable of preventing the adherence of infected red blood cells to dendritic cells using the effect of inhibition of maturation of immature dendritic cells is new (Article 33(2) PCT) and involves an inventive step (Article 33(3) PCT). The prior art does not give any indication that the CD36 and CD51 receptors are involved in the maturation of immature dendritic cells.

Item VIII

Certain observations on the international application

1. Claims 6, 7, 35, 36, 41, 42, 45, 52 are not clear (Article 6 PCT) because they refer to a part of the description to define technical features of the invention (see PCT Guidelines III-4.10).
2. The anti-CD36 antibody clone SMØ and the anti-CD51 antibody clone 13C2 is not sufficiently disclosed in the description because it appears that the supplying company does not exist anymore
3. Claims 6, 7, 35, 36, 41, 42, 45, 52 are not supported by the description (Article 6 PCT) and not sufficiently disclosed for the entire breadth of the scope of said claims (Article 5 PCT). Said claims refer to lists of antibodies which were selected for their binding specificity to the respective cell surface antigens but not for their immunosuppressant effect on immature dendritic cells. The present application does not provide any substantive support that said antibodies are agonists of the CD36 and CD51 receptors.
4. Claim 4 is not sufficiently supported by the description (Article 6 PCT) and not sufficiently disclosed. In the examples of the description the only anti-CD51 antibody which was shown to have the desired immunosuppressant is designated as clone 13C2 of the company Immunocontact (see also section 3 above). It appears that this company does not exist anymore under the address given. Therefore the skilled person would not have been able to carry out the invention.

5. Claim 9 is not clear (Article 6 PCT) because the term antigenic material is too vague and applies also to the agonist used.
6. Claim 15 is not clear (Article 6 PCT) and lacks an essential feature. The method as given in claim 15 determines the effect on dendritic maturation by an unknown molecule. As the interaction with the CD36 and CD 51 receptors is possibly not the only mechanism by which maturation of dendritic cells can be inhibited, the steps of claim 26 must be included in claim 15 in order to assure that agonists to the receptors CD36/CD51 are detected.



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- (71) Applicant (*for all designated States except US*): **ISIS INNOVATION LTD. [GB/GB];** Ewert House, Ewert Place, Summertown, Oxford OX2 7BZ (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **ROBERTS, David, John [GB/GB];** National Blood Service Oxford Centre, John Radcliffe Hospital, Headington, Oxford OX3 9DU (GB). **URBAN, Britta, Christina [DE/GB];** Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DS (GB).
- (54) Agent: **BOULT WADE TENNANT;** Verulam Gardens, 70 Gray's Inn Road, London WC1X 8BT (GB).
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WO 01/02005 A2

(54) Title: **INDUCTION OF IMMUNE TOLERANCE**

(57) Abstract: Methods and compositions for the induction of immune tolerance in mammalian antigen presenting cells such as dendritic cells, macrophages, monocytes and B-lymphocytes are described. Such methods and compositions involve the use of agonists of the cell surface receptors CD36, CD51, thrombospondin receptors and/or the β -integrins which when exposed to an antigen-presenting cell such as a dendritic cell are able to inhibit maturation therein. Thus, the cells' ability to promote an immune response is inhibited. Tolerance to a specific antigen can be induced in antigen-presenting cells by exposure to one or more of the aforesaid agonists and the antigen. Thus, cell preparations can be prepared for administration to humans where tolerance to a specific antigen or antigens needs to be induced, for example in the case of allograft or xenograft transplants or in autoimmune disease.

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INDUCTION OF IMMUNE TOLERANCE

5 The invention relates to the field of immune suppression and, in particular, to the identification of molecules which act as agonists of the cell surface
10 receptors CD36, CD51 and thrombospondin receptors expressed on mammalian dendritic cells and other antigen-presenting cells, to ex vivo and in vivo uses of such molecules for inducing peripheral immune
15 tolerance in mammals, to identification of molecules which inhibit the state of immune tolerance induced in a human by the binding of red blood cells infected with the malarial parasite to dendritic cells and to
20 in vivo uses of such molecules in treating malaria.

15 Dysfunction of the immune system has been shown to play a role in the initial development and further progression of many human diseases. Impaired immune function can result in inability to fight infection or to destroy malignant cells as they develop within the
20 body. Other diseases are caused because the immune system mounts an inappropriate response to a particular antigen. This inappropriate response might be to an external antigen resulting in atopic disease such as hay fever, asthma, eczema, coeliac disease and
25 the like or to the body's own antigens resulting in auto-immune disease. For example both the non-organ specific auto-immune diseases, such as systemic lupus erythromatosis and rheumatoid arthritis and the organ specific auto-immune diseases such as auto-immune
30 haemolytic anaemia and idiopathic thrombocytopenic purpura are associated with an inappropriate T-cell response to self-antigens.

35 Other auto-immune diseases where the antigen has been defined include auto-immune connective tissue syndromes, insulin dependent diabetes mellitus and auto-immune thyroid disease. Diseases where the antigen is less well defined include auto-immune skin

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diseases such as eczema, psoriasis, alopecia areata and vitiligo, auto-immune diseases of the gastro-intestinal system such as inflammatory bowel disease and auto-immune hepatitis, auto-immune diseases of the nervous system such as multiple sclerosis and myasthenis gravis and auto-immune diseases of the kidney such as glomerulonephritis.

In view of the diseases associated with inappropriate immune response, particularly T-cell response, it is highly desirable to develop pharmaceuticals which are able to damp down certain of the body's immune defence mechanisms in order to alleviate the distressing symptoms associated with these diseases.

As well as treatment of diseases specifically associated with a mal-function of the immune system, down-modulation of immune mechanisms is desirable in circumstances where a recipient is exposed to allo-antigens or xeno-antigens for therapeutic purposes such as recipients of allogeneic or xenogeneic transplants. An allogeneic response in the case of allogeneic bone marrow transplantation or donor lymphocyte infusion might be avoided if one could induce a state of peripheral immune tolerance against donor cells in the recipient. Other examples of situations where down-modulation of immune mechanisms might be desirable include haemolytic disease of the new born, neo-natal allo immune thrombocytopenia or the therapeutic administration of antigenic substances such as blood products e.g. factor VIII, or any other therapeutic or prophylactic agent likely to induce an unwanted cellular immune response.

A cellular immune response is mediated by T-lymphocytes which are activated by antigen presenting cells, the most important of which are dendritic cells, which present antigen and activate memory T-cells and naive T-cells. Dendritic cells become

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potent antigen-presenting cells when exposed to an immune stimulus and thereafter are described as "mature". Maturation confers enhanced ability to stimulate T-cells and a reduction in pinocytosis and phagocytosis compared with immature cells. Furthermore, maturation is accompanied by enhanced cell surface expression of HLA Class I and class II molecules as well as adhesion molecules, including CD54 and co-stimulatory molecules such as CD80, CD86 and the cell-surface marker CD83. Maturation of dendritic cells is also accompanied by the secretion of cytokines such as TNF α and IL12p70. The secreted cytokines have an autokrine effect on dendritic cell maturation itself and parakrine effects on interacting T-cells.

Immature dendritic cells present the cell surface antigens CD36 and CD51 (α_v) (part of the vitronectin receptor $\alpha_v\beta_3$). CD36 and integrin heterodimers $\alpha_v\beta_3$ or $\alpha_v\beta_5$ can be cross-linked by the soluble bridging molecule thrombospondin (TSP). Through studies of malarial infection the present inventors have discovered that dendritic cell maturation on exposure to an immune stimulus, for example, lipopolysaccharide (LPS), can be inhibited by molecules which bind to CD36 or to CD51 or both via the bridging molecule TSP and which act as agonists thereto.

This discovery is based on the inventors' initial observations that red blood cells infected with the malarial parasite *Plasmodium falciparum* adhere to dendritic cells via CD36 and/or TSP/CD51 (see Figure 1) and are able to inhibit the maturation thereof on exposure to LPS.

Plasmodium falciparum is one of the most successful human pathogens for which virulence factors remain poorly defined, although adhesion of infected erythrocytes to venular endothelium has been associated with some of the symptoms of severe

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disease. Immune responses are unable to prevent symptomatic infections throughout life and immunity to severe disease develops only slowly during childhood. Understanding the obstacles to the development of protective immunity is crucial for rational approaches to prevent the disease.

Specific immunity to malaria has been attributed to cytotoxic lymphocytes active against the liver stage of infection or to antibodies reacting against blood stage antigens. Antigenic diversity, clonal antigenic variation and T-cell antagonism may contribute to evasion of the protective and parasitocidal host responses.

Furthermore, it is known that *Plasmodium falciparum*-infected erythrocytes adhere to endothelial cells and it has been widely assumed that this adhesion has evolved to mediate sequestration of parasites to endothelial cells in the peripheral tissues and so reduce their destruction by splenic macrophages.

The present inventors have now identified a further mechanism by which the malarial parasite prevents the infected host from mounting an effective immune response and preventing recurrence of the disease.

Specifically, the inventors have observed that human erythrocytes which are infected with *Plasmodium falciparum* are capable of adhering to human dendritic cells and that immature dendritic cells exposed to infected erythrocytes are no longer able to mature into full antigen-presenting cells or to stimulate T-cell proliferation, when subsequently exposed to an immune stimulus. However, this state of immune tolerance is not observed when the dendritic cells are exposed to uninfected erythrocytes, uninfected erythrocyte lysate, infected erythrocyte lysate, parasite-conditioned medium or a crude pigment

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preparation derived from infected erythrocytes. Further, the effect is not observed when dendritic cells are exposed to erythrocytes infected with a *Plasmodium falciparum* strain T9/96 which is known not to be able to adhere to endothelial cells (Gardner et al (1996) Proc. Natl. Acad. Sci. USA 93 pp 3503-3508). This particular strain is not able to induce expression on the surface of infected erythrocytes of the parasite-derived protein pf-EMP-1 which is known to undergo clonal antigenic variation and is thought to be the mediator of adherence to endothelial cells. It has been reported that most parasite lines and clones adhere to the known cell-surface receptors CD36 and via TSP to CD51/61 ($\alpha_v\beta_3$). It is also known that pf-EMP-1 can bind to CD36. (see WO 96/33736).

The present inventors have now shown that CD36 and CD51 influence the process of dendritic cell maturation and that agonists thereof, including the malarial parasite derived protein pf-EMP-1, antibodies specific for CD36 and CD51, negatively charged phospholipids and apoptotic cells, are able to inhibit dendritic cell maturation in response to an immune stimulus. These agonists reduce the ability of the dendritic cells to stimulate T-cell proliferation in response to an antigen to a level which is lower than cells which have not been exposed to an immune stimulus at all. Thus, agonists of CD36 and CD51 can induce a state of immune tolerance.

It follows that agonists of CD36 and CD51 would be useful for the treatment of the types of autoimmune disease described above where an over-reaction of the host immune system is responsible for the symptoms. Further the inventors have found that dendritic cells may be treated by CD36 and/or CD51 agonists *in vitro* together with an antigen specific to the immune-response manifested in the auto-immune disease in question. Thus, tolerance may be induced to a

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specific antigen so that, when the dendritic cells are reintroduced into the host, further auto-immune reaction is avoided or substantially reduced. In addition CD36 and CD51 agonists are useful for
5 inducing a state of immune tolerance in both host and donor dendritic cells where bone marrow transplantation or lymphocyte infusion is contemplated. The feasibility of such treatment is demonstrated herein *in vivo* in mice. The ability to
10 inhibit maturation of dendritic cells can be demonstrated *in vitro* so that molecules which act as CD36 or CD51 agonists can be easily identified in a high throughput screening assay.

As used herein the term "agonist" means a
15 composition, molecule, cell or a component thereof which induces the same response when interacting with a receptor as the naturally-occurring ligand for that receptor.

In accordance with a first aspect the invention
20 provides a method of identifying a molecule which is an agonist of cell surface receptor CD36 and/or CD51 as expressed by mammalian dendritic cells which method comprises:

- 25 a) exposing immature mammalian dendritic cells to the molecule to be tested,
- b) exposing said immature dendritic cells to an immune stimulus and
- 30 c) determining the degree of maturation manifested by said dendritic cells,

wherein impaired maturation in response to the immune
35 stimulus is an indication that said molecule under test is a CD36 and/or CD51 agonist.

Preferably, the method is performed using human

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dendritic cells. As used herein the term dendritic cells means cells that present antigen to and activate lymphocytes and which are distinguished by their ability to activate, not only memory T-cells but also naive T-cells. Dendritic cells for use in the method of the invention may be derived by cultivation of adherent peripheral blood mononuclear cells with the addition of Granulocyte-Macrophage Stimulating Factor and Interleukin-4 for about 6 to 10 days. Such dendritic cells can be characterised by their level of expression of the cell-surface markers HLA Class I and II (high), CD11 c (high), CD3 and CD19 (negative), CD14 (low) and CD86 (high). These markers distinguish them from B-cells which are positive for CD19, T-cells which are positive for CD3 and macrophages which are CD14 high and CD86 low. (See Banchereau et al, (1998) *Nature* 392, 245-252). Antibodies to HLA Class I, HLA class II, CD14, CD3, CD19 and CD86 useful for identifying immature dendritic cells are commercially available as indicated in Table 1 below.

Dendritic cells which may be used in the method of the invention can also be derived directly from circulating peripheral blood mononuclear cells or by culture of CD34+ stem cells as described by Caux et al (1996) *J. Exp. Med.* 184:695-706 and Arrishi et al (1999) *Blood* 93:2244-2256.

There are various ways in which maturation of dendritic cells in response to an immune stimulus, may be measured. On maturation the dendritic cells become potent antigen presenting cells. As aforesaid maturation is accompanied by enhanced cell surface expression of HLA Class I and II molecules such as HLA DR, adhesion molecules such as CD54 and co-stimulatory molecules such as CD40, CD80, CD86 and CD83 which is a specific marker for mature dendritic cells. Thus, examination of the cell's antigen presenting ability, for example variety of antigens and/or level of

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expression, is one way of determining whether maturation has occurred or whether it has been inhibited by the test molecule. Preferably, following immune stimulation, the level of expression of the HLA Class I and II molecules and/or adhesion molecules and/or co-stimulatory molecules is measured. In one embodiment maturation of dendritic cells is detected by measurement of the level of expression of two or more of the cell-surface antigens HLA DR, CD54, CD40, CD83 and CD86 whose level of expression is particularly enhanced. Preferably, the level of expression of all of the above in response to an immune stimulus is measured. Optionally the expression level of CD80 may also be measured.

Methods by which the expression of a cell-surface antigen may be quantified are well-known to those skilled in the art. The commonly used method is to apply an antibody specific for the antigen in question to the antigen-presenting cells which has been labelled to give a quantifiable detectable signal. Suitable labels are well-known to those skilled in the art and include radioactive labels, enzyme labels, fluorescent labels, metallic particles and the like. Antibodies suitable for carrying out the screening method of the present invention, as well as a commercial source, are shown in Table 1 below:

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TABLE 1

5		<u>Antigen</u>	<u>Antibody</u>	<u>Source</u>
		HLA DR	BF-1	Serotec
		HAL Class 1	W32/6	ATCC HB-95
10		CD14	Tük4	DAKO
		CD54	6.5B5	DAKO
		CD40	LOB7/6	Serotec
		CD80	BB1 or DAL 1	Serotec
		CD83	HB15a	Serotec
15		CD86	BU63	Serotec
		CD3	OKT3	ATCC CRL-8001
		CD19	HD37	DAKO
		CD36	clone 89	Serotec
			clone SMØ	Immunocontakttec
20		Serotec: 22 Bankside, Station Approach, Kidlington, Oxford, UK DAKO Ltd: 16 Manor Courtyard, Hughenden Avenue, High Wycombe, Bucks HP13 5RE		
		Immunokontakt: Centro Nord-Sud, CH-6934 Bioggio, Switzerland, Peprotec: 23 St. James Square, London		
25		SW9Y 4JH, UK, ATCC: 10801 University Boulevard, Manassas, VA 20110-2209; USA, Sigma: Sigma Alderich Company Ltd: Fancy Road, Poole, Dorset, BH12 4QH, UK, Schering-Plough: Schering-plough House, Shire Park,		
30		Welwyn Garden City, Herts, AL7 1TW.		
		As an alternative to measuring the level of cell surface antigen to determine whether or not dendritic cell maturation has occurred, it is possible to measure the cell's ability to induce T-cell		
35		proliferation. This is inhibited by agonists of CD36 or CD51. Dendritic cells which have been exposed to the molecule to be tested and to an immune stimulus		

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may be exposed to T-cells, for example allogeneic lymphocytes in a mixed lymphocyte reaction (MLR) with the T-cell receptor. The T-cells respond by growing and dividing, something which can easily be measured using methods well-known to one skilled in the art. For example, growth and division can be assessed visually using a light microscope to observe clumps of dividing cells. Alternatively, cell proliferation can be quantified using a suitably labelled metabolite, for example tritiated thymidine, which is incorporated into the cell's DNA.

A yet further alternative for determining the degree of dendritic cell maturation is to measure the level of secretion of cytokines such as TNF α , IL2p70 or IL10.

For example IL12p70 is secreted by mature cells but not by immature cells. The level of TNF α secretion is reduced in immature as opposed to mature cells. Kits are commercially available for detection and quantitation of all of the above cytokines. (see Examples). Preferably, the levels of TNF α , IL12p70 and IL10 secretion are measured.

In the screening method of the invention a variety of immune stimuli may be used. Suitable examples are lipopolysaccharide (available from Sigma), TNF α (available from Peprotec) and monocyte conditioned medium (MCM) the preparation of which is described by Romani et al (1996) J. Immunol. Methods, Sep 27; 196(2):137-51. Another suitable immune stimulant is CD40L which is expressed from plasmids having the ATCC Accession No's 79812, 79813, 79814 or 79815. The plasmids may be expressed in mouse fibroblasts STO (ATCC-CRL-1503).

It will be understood that TNF α would not be used as both immune stimulant and indicator of cell maturation in the same assay.

In a particular embodiment of the method of the

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invention immature dendritic cells (about 10^6) are exposed in duplicate to various concentrations of the test molecule for about 3 to about 12 hours in a multiwell plate. The test compound is prepared in a suitable diluent which is not toxic to the dendritic cells such as tissue culture medium, PBS, water or a suitable non-toxic organic solvent, if appropriate. The duplicate wells are subsequently exposed to LPS (about 500 ng/ml) or left untreated for about 48 hours. For each concentration of the compound and time of exposure, the surface expression of the molecules identified above is compared with the surface expression on immature dendritic cells exposed to the test compound as well as untreated immature dendritic cells. The increase in cell surface expression is evaluated using indirect immunofluorescence and FACScan analysis. A compound is a candidate for further evaluation if the surface expression on dendritic cells of at least two cell-surface antigens is not increased by addition of the immune stimulant, LPS.

Preferably, molecules identified as potential CD36 or CD51 agonists by the method of the invention will be subject to further evaluation. For example, if surface expression of lineage-specific molecules has been used to determine the degree of maturation it would be usual to check whether the compound can also prevent immune-stimulated dendritic cells from inducing proliferation of T-cells and visa versa. The ability of the molecule to vary cytokine secretion could also be tested. In addition direct binding of the candidate molecule to CD36, CD51 or TPS should also be confirmed. This latter confirmation may be easily obtained by applying a sample of the candidate molecule to a purified sample of CD36, CD51 or TPS. Purified CD36 may be prepared as described by Tandon et al (1989) The Journal of Biological Chemistry, 264

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pp 7570-7575. Purified CD51 may be prepared as described by Smith et al, (1990), Journal of Biological Chemistry, 265, 11008-11013 and purified TSP may be prepared as described by Silverstein et al
5 (1985), Journal of Clinical Investigation, 75, pp 2065-2073.

Tests to detect binding of the test molecule are conveniently carried out by immobilizing the CD36, CD51 or TSP to a solid surface, for example the
10 surface of a well of a microtitre plate. Methods of immobilization of protein molecules on such surfaces are well-known to those skilled in the art. The test molecule identified as a CD36 or CD51 agonist is then applied to the immobilized protein. Following removal
15 of unbound test molecule the presence of bound molecule is directly detected. This may be achieved in a number of ways depending on the chemical or biochemical characteristics of the test molecule.

For example where the test molecule is a protein
20 it would be usual to detect binding with a labelled antibody to that protein. If the test molecule is a non-antigenic small molecular weight compound then the compound itself may be radioactively labelled for detection.

25 The molecule whose activity is to be tested in the method of the invention may have any type of molecular structure. For example, it may be a protein, a peptide, an amino acid, DNA, RNA, PNA, a nucleotide or a nucleoside, or a low molecular weight
30 compound. It may be a molecule having known pharmacological or biochemical activity or a molecule with no such known activity and may be a novel molecule. The method of the invention is suitable for testing entire libraries of molecules, for example
35 libraries such as would be created by combinatorial chemistry. Indeed, all the embodiments of the screening method above may be adapted for an automated

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high throughput compound screen.

Using the method of the invention the present inventors are able to confirm that the *Plasmodium falciparum* derived protein pf-EMP-1 is an agonist of both CD36 and CD51. In particular a fragment of pf-EMP-1 known as CIDR/A4 which comprises the CD36 binding domain is an agonist of CD36. CIDR/A4 is described by Smith et al (1998) Molecular and Biochemical Parasitology, 97, pp 133-148 and comprises amino acids 402 to 846 of pf-EMP-1 as shown in Figure 2.

Antibodies which bind CD36 and CD51 have also been identified as having agonist activity and are capable of inhibiting the maturation of dendritic cells. Thrombospondin is also an agonist of CD51. The present invention is also directed to any individual molecule identified as an agonist of CD36 or CD51 by the methods described herein.

The assays of the invention have allowed the inventors to make the further observation that apoptotic cells, the natural ligand of CD36, are also able to inhibit dendritic cell maturation in response to LPS. This is yet further evidence of the role of CD36 in modulating immune response.

In accordance with a second aspect the invention provides a pharmaceutical composition suitable for inducing immune tolerance in a mammal which comprises an agonist of the cell surface receptor CD36 and a pharmacologically acceptable carrier or diluent. The CD36 agonist may be a molecule identified by the method described above. Agonists which are suitable for incorporation into a pharmaceutical composition in accordance with the invention for the treatment of humans include antibodies with an affinity for an epitope of CD36, in particular an antibody which blocks the binding domain on CD36 for pf-EMP-1. Monoclonal antibodies specific for CD36 which are

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designated "clone 89" and "clone SMΦ" and which are commercially available from Serotech or Immunocontact (details above) are suitable for use in the pharmaceutical compositions of the invention.

5 Other commercially available CD36 antibodies which may be included in pharmaceutical compositions are listed in Appendix 1. It is contemplated that compositions comprising antibodies bispecific against CD36 and CD51
10 will be useful for inhibiting dendritic cell maturation.

Other agonists suitable for inclusion in pharmaceutical compositions are all variants of the *Plasmodium falciparum* pf-EMP-1 or fragments of such proteins which comprise the binding domain for CD36.
15 A particular example is the fragment CIDR/A4 described herein comprising amino acids 402 to 846 of pf-EMP-1. (Figure 2).

Pharmaceutical compositions comprising a bispecific CD36 antibody and the CIDR/A4 fragment are
20 also contemplated in accordance with the invention.

Yet another agonist suitable for inclusion in a pharmaceutical composition are negatively charged phospholipids such as phosphatidylserine containing liposomes which have also been shown to bind to CD36
25 and other cellular receptors of immune cells.

Yet another agonist suitable for inclusion in a pharmaceutical composition are apoptotic cells.

In a third of its aspects the invention provides a pharmaceutical composition suitable for inducing
30 peripheral immune tolerance in a mammal which comprises an agonist of the cell surface receptor CD51 as expressed by mammalian dendritic cells and a pharmacologically acceptable carrier or diluent. As with CD36 acceptable agonists are antibodies,
35 preferably monoclonal antibodies, directed against an epitope of CD51. Particularly suitable are antibodies blocking the binding domain of CD51 for the bridging

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molecule TSP. Antibodies suitable for incorporation in a pharmaceutical composition in accordance with this aspect of the invention are commercially available and set out in Appendix 2.

5 Thrombospondin (TSP) is also suitable for incorporation into a pharmaceutical composition as a CD51 agonist. Preferably, such compositions also include the *Plasmodium falciparum* protein pf-EMP-1 or a fragment thereof incorporating the thrombospondin
10 binding domain of pf-EMP-1.

 As with CD36, negatively charged phospholipids such as phosphatidylserine are also suitable for incorporation as CD51 agonists in pharmaceutical compositions of the invention as well as apoptotic
15 cells.

 Pharmaceutical compositions in accordance with the second and third aspects of the invention are useful for the treatment of autoimmune diseases associated with inappropriate dendritic cell
20 maturation and T-cell proliferation such as systemic lupus erythromatosis, rheumatoid arthritis, autoimmune haemolytic anaemia or idiopathic thrombocytopenic purpura. Vehicles suitable for delivery of pharmaceutically active substances are known to those
25 skilled in the art, especially those for delivery of pharmaceutically active proteins.

 In accordance with a fourth aspect of the invention there is provided a method of treating mammalian dendritic cells *in vitro* to induce immune
30 tolerance therein which comprises exposing said cells to an agonist of cell surface receptors CD36 and/or CD51 as expressed on mammalian dendritic cells. The invention also relates to preparations of cells so treated. Suitable agonists are any of those agonists
35 described above or any molecule or substance identified by the screening method described herein.

 Treatment of dendritic cells *ex-vivo* with an

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agonist of CD36 and/or CD51 is beneficial in many therapeutic applications as described hereinafter. For example, in the case of bone marrow transplantation or lymphocyte infusion recipient cells removed from the body are treated with agonists as described above to induce a state of immune tolerance therein. The treated cells are then re-introduced to the body before or simultaneously with the donor cells and the risk of allogeneic reaction is thereby reduced or eliminated. It is contemplated that dendritic cells of the donor may also be treated with a CD36 and/or CD51 agonist to induce immune tolerance. The donor may be allogeneic or xenogeneic.

The present inventors have demonstrated that in mice tolerance to foreign antigens can be achieved by exposure of dendritic cells from one mouse strain, *ex-vivo*, to a CD51 agonist followed by introduction of the treated cells into another strain of mice. Thus such therapy is expected to be applicable to humans.

In addition the present inventors have shown using a fragment of the α -subunit of the human acetylcholine receptor that immature dendritic cells treated with a CD36 agonist can be "modulated" to induce tolerance against a specific antigen by subsequent *ex-vivo* exposure to that antigen. Once reintroduced *in vivo*, immune response to that antigen is reduced or avoided. Thus, dendritic cells may be removed from a patient suffering from an auto-immune disease, for example, and exposed to a CD36 and/or CD51 agonist and an antigenic molecule associated with the auto-immune disease in question and the dendritic cell preparation, with or without maturation, reintroduced into the patient. Alternatively, this method may be used to induce tolerance to a particular allo or xeno-antigen or other therapeutic substance which is likely to induce an unwanted immune response,

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such as a blood product like factor VIII.

Thus, the invention includes preparations of dendritic cells tailored to the treatment of a particular auto-immune disease by exposure to an agonist of CD36 and/or CD51 and the specific auto-antigen associated with the disease and cell preparations tolerant to other antigens likely to generate an unwanted immune response.

It follows from the inventor's observations concerning inhibition of maturation of dendritic cells with agonists of CD36 and CD51 that a similar effect will be observed with agonists of thrombospondin receptors in general and with other antigen-presenting cells of the immune system which also express CD36 and CD51 and thrombospondin receptors such as macrophages, B-lymphocytes and monocytes.

Thus, in accordance with a fifth aspect the invention provides a method of identifying a molecule which is an agonist of cell surface receptors CD36 and/or CD51 and/or a thrombospondin receptor as expressed on antigen-presenting cells of the mammalian immune system which method comprises:

- a) exposing mammalian antigen-presenting cells to the molecule to be tested,
- b) exposing said cells to an immune stimulus and
- c) determining the response to said immune stimulus by said cells,

wherein an impaired response compared to the response in the absence of said test molecule is an indication that said molecule under test is a CD36 and/or CD51 agonist or an agonist of a thrombospondin receptor.

Preferably, the response that is measured is maturation of said antigen presenting cell. Such a

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screening method may be carried out using the general methodology already described herein for dendritic cells. For example, monocytes can be purified from peripheral blood by adherence of PBMC to plastic dishes. Non-adherent cells are removed and the adherent cells can be detached by incubation with EDTA in PBS. Contaminating lymphocytes are depleted with the aid of magnetic heads and antiCD3 and antiCD19 monoclonal antibodies. Macrophages may be generated by culturing monocytes, which have been isolated as described above, in RPMI 1640 supplemented with M-CSF for six days. β -lymphocytes can be isolated from blood by virtue of their non-adherence to plastic petri dishes. The non-adherent cells are subjected to depletion of contaminating monocytes and T-cells by exposure to magnetic heads and antiCD14 and antiCD3 monoclonal antibodies.

Once isolated the antigen presenting cells are exposed to a substance to be tested for agonist activity against CD36, CD51 or a thrombospondin receptor and the degree of activation of said cells is measured. As with dendritic cells, activation may be determined by measuring the levels of secretion of various cytokines, or by testing ability of said antigen presenting cells to stimulate T-cell proliferation. In a preferred embodiment the increased expression of certain cell surface receptors is used as a measure of activation. In the case of monocytes and macrophages activation is accompanied by an increase in surface expression of HLA-DR, CD54 and CD86 which is measured in the manner described above, preferably with the use of a monoclonal antibodies to HLA-DR, CD54 and CD86. B-cell activation is determined by measuring the level of cell surface expression of HLA-DR, CD86 and CD40. The expression may be detected using antibodies to these cell surface receptors. Such as clone BF1, Serotech (HLA DR) clone

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BU63, Serotech (CD86) and clone TRAP1 (Pharmingen) (CD40).

5 The invention also further relates to uses of an agonist as identified above using said antigen-presenting cells for treatment of any of the auto-immune diseases listed above and for inducing immune tolerance in said antigen presenting cells ex-vivo as well as to antigen-presenting cell preparations which have been treated with a CD36 and/or CD51 agonist
10 and/or thrombospondin receptor agonist and optionally an antigenic material.

The invention also relates to pharmaceutical compositions comprising an agonist of a thrombospondin receptor, for example $\alpha_v\beta_3$ or $\alpha_v\beta_5$, with a
15 pharmaceutically acceptable carrier or diluent suitable agonists include antibodies to the thrombospondin binding domain of said receptor, for example any of the antibodies listed in Appendix 3.

Other suitable agonists include negatively
20 charged phospholipids such as phosphatidylserine containing liposomes.

It is a further conclusion from the work of the present inventors that agonists of β -integrin associated with the cell surface receptor CD51 as
25 expressed on the surface of antigen-presenting cells of the mammalian immune system, will also be useful for inducing immune tolerance. The invention in a sixth aspect thus, further relates to methods of identifying β -integrin agonists by any of the
30 procedures described above and to uses of β -integrin agonists, as defined above, for any of the medical uses which are described herein.

In a seventh aspect the present invention further relates to uses of apoptotic cells as a medicament for
35 inducing immune tolerance in antigen-presenting cells, preferably dendritic cells and to pharmaceutical compositions comprising those cells in a suitable

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carrier or diluent. Apoptotic cells are suitable for delivering tissue specific antigens including major and minor histocompatibility antigens to dendritic or other antigen-presenting cells. Delivering antigens in this way allows delivery of unknown antigens or antigens where the class II restricted epitope(s) are not defined. The tissue origin of the apoptotic cell may be varied depending upon the application. For example, it is preferred for the apoptotic cell to be of the same tissue type as any cell bearing an antigen to which tolerance is to be induced.

In accordance with an eighth aspect the invention further relates to the use of negatively charged phospholipids for inducing immune tolerance in antigen presenting cells. Said immune tolerance may be induced by treatment of said antigen presenting cells, for example dendritic cells, with said negatively-charged phospholipid either ex-vivo by the methods described herein or by administration of the phospholipid to a patient by any of the conventional administration routes known to those skilled in the art. A preferred form of composition is liposomes comprising the negatively charged phospholipid. A preferred phospholipid is phosphatidylserine.

Since the inventions of the present application were developed following the basic observation that *Plasmodium falciparum* infected erythrocytes adhere to dendritic cells and inhibit the maturation thereof it follows that molecules which block or inhibit such adherence may be useful as pharmaceuticals in the clinical management of malaria, in particular molecules which inhibit adherence of parasite-infected erythrocytes to CD36 or TSP.

Thus, in accordance with a ninth aspect of the invention a method comprising the following steps is used to identify a molecule capable of preventing adherence of erythrocytes infected with a malarial

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parasite to human dendritic cells:

(a) exposing a purified preparation of CD36 or
TSP to:-

- 5 (i) the molecule to be tested and
 (ii) parasitised human erythrocytes

either consecutively or simultaneously and

- 10 (b) determining the level of adherence of said
 parasitised erythrocytes to said CD36 or TSP

15 wherein a reduction in the level of adherence to CD36
 or TSP in the presence of the test molecule compared
 to the level of adherence in the absence of said test
 molecule is an indication that said molecule is
 capable of preventing the adherence of erythrocytes
 infected with the malarial parasite to human dendritic
 cells.

20 The erythrocytes may be infected with *Plasmodium*
falciparum or another *Plasmodium* species. Suitable
falciparum strains include ITO/A4 or ITO/C24 which may
be derived as described by Roberts et al (1992) Nature
357 pp 689-692 or Malayan Camp (MC) which may be
25 obtained as described by Roberts et al (1985) Nature
 318:64-66.

 A suitable format for carrying out a screening
method as described above is to immobilize the
purified CD36 or TSP onto a solid surface.
Preferably, immobilization is secured by adsorption of
30 the protein molecules to a plastic surface such as a
petri dish. Parasitised erythrocytes suspended in a
suitable binding medium are added to the adsorbed CD36
or TSP and incubated for a period sufficient to allow
adherence, for example, about 1 hour. Thereafter the
35 binding medium and any non-adhered erythrocytes are
removed and a suitable erythrocyte stain for example,
Giemsa, added to the petri dish. Adhered erythrocytes

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may be quantified by counting under a light microscope. Alternatively, depending on the stain used, erythrocyte adherence may be quantified by spectrometry, fluorescence microcopy and the like.

5 In a tenth aspect the invention provides a method of identifying a molecule capable of preventing the adherence of red blood cells infected with a malarial parasite to human dendritic cells which comprises:

- 10 a) exposing immature human dendritic cells to the *Plasmodium falciparum* protein pf-EMP-1 or an active binding domain thereof in the presence or absence of the molecule to be tested,
- 15 b) exposing said immature dendritic cells to an immune stimulus and
- c) determining the degree of maturation manifested by said dendritic cells,
- 20 wherein any maturation of said dendritic cells in the presence of the test molecule over and above that manifested in the absence of said molecule is an indication that said molecule is capable of preventing
- 25 adherence of red blood cells infected with a malarial parasite to human dendritic cells.

 Maturation of dendritic cells may be measured by any of the methods already described herein. Suitable immune stimulants include LPS, TNF α , CD40L and

30 monocyte conditioned medium (MCM). Preferably the pf-EMP-1 preparation for use in the method is that designated in pf-EMP-1 A4var as described by Smith et al (see before) and having the Genbank Accession No. L42244. The fragment CIDR/A4 may also be used.

35 In a further aspect the invention provides for use of molecules identified by the aforementioned methods which inhibit infected erythrocyte adherence

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to dendritic cells in pharmaceutical compositions for the treatment of malarial infection.

Based on the present inventors' observations it is further contemplated that a modified CIDR region of the pf-EMP-1 A4 variant protein could be incorporated in a multisubunit vaccine against *falciparum* malaria. This would induce blocking antibodies against the CD36 binding domain of pf-EMP-1 variant proteins so that the immune responses against other proteins are not inhibited.

All documents cited in this application are incorporated herein by reference.

The invention will now be further described with reference to the following Figures and Examples.

FIGURE 1 shows schematically the molecular basis for the binding of Plasmodium falciparum infected red blood cells to CD36 and TSP on the surface of dendritic cells;

FIGURE 2 shows the amino acid sequence of the pf-EMP-1 fragment CIDR/A4;

FIGURE 3 shows the increase in surface expression of dendritic cell marker antigens HLA DR, CD54, CD40, CD80, CD83 and CD86 following immune stimulation after exposure to (a) LPS matured dendritic cells, (b) dendritic cells matured with LPS, with and without prior exposure to RBC, (c) dendritic cells matured with LPS with and without prior exposure to parasite lysate and (d) dendritic cells matured with LPS with and without prior exposure to intact ITO/A4 infected RBC;

FIGURE 4; (A) shows the absolute binding of erythrocytes infected with parasite lines ITO/A4, ITO/C24, MC and T9/96 to CD54, CD56, and TSP (a,c,e,g)

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and (B) shows the increase in surface expression of LPS matured dendritic cells compared with dendritic cells exposed to the respective parasite line prior to maturation (b,d,f,h);

5

FIGURE 5 shows transmission electron micrographs illustrating the interaction of dendritic cells with (a) ITO/A4 infected erythrocytes and (d) non-adherent T9/96 infected erythrocytes;

10

FIGURE 6 shows dendritic cell stimulation of T-cell proliferation (a) induced by immature dendritic cells (■), LPS-matured dendritic cells (□) and dendritic cells co-cultivated with intact ITO/A4 infected erythrocytes (▼) prior to maturation, primary CD4+ T-cell responses to parasite lysate (b) and to keyhole limpit haemocyanin (c) induced by LPS-matured autologous dendritic cells (□,○) and autologous dendritic cells co-cultivated with intact ITO/A4 infected erythrocytes (■,●) prior to maturation;

15

20

FIGURE 7 shows the effect of monoclonal antibodies against CD36 and CD51 on maturation of dendritic cells represented graphically as relative increase in surface expression of dendritic cells matured with LPS compared with immature dendritic cells;

25

FIGURE 8 shows the effect of monoclonal antibodies against CD36 and CD51 on dendritic cell maturation as a FACscan output;

30

FIGURE 9 shows further results of experiments with apoptotic cells (a) output of FACscan, (b) staining with potassium iodide to exclude dead cells, (c) proliferation of allogenic T-cells stimulated by increasing numbers of immature dendritic cells, (◆) LPS-matured dendritic cells (▲) or dendritic cells

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exposed to apoptotic dendritic cells and then matured with LPS(■).

5 FIGURE 10 shows the effect of apoptotic neutrophils on the maturation of dendritic cells;

FIGURE 11 shows results of a T-cell proliferation assay including antigen specific T-cell proliferation. (a) Proliferation of allogeneic T-cells. (b) proliferation of KLH specific CD4+CD45RO- autologous T-cells (c, d) proliferation of the T-cell clone TB-2 specific for the human Acetylcholine Receptor α -subunit in response to polypeptide (c) or peptide (d). Stimulator dendritic cells were treated as follows:

10 immature DC alone (*) or matured with LPS (□);
dendritic cells exposed to irrelevant antibodies with (O) or without (●) antigen and then matured with LPS;
dendritic cells exposed to antiCD36 antibody with (v) or without (▼) antigen and then matured with LPS;
20 dendritic cells exposed to antiCD51 antibody with (Δ) or without (▲) antigen and then matured with LPS;
dendritic cells exposed to antiCD36 and antiCD51 antibody with (◇) or without (◆) antigen and then matured with LPS.

25 FIGURE 12 shows secretion of cytokines $\text{TNF}\alpha$, IL12p70 and IL10 by dendritic cells exposed to an antiCD36 antibody or to apoptotic dendritic cells and respective controls;

30 FIGURE 13 shows *in vitro* maturation of mouse dendritic cells following exposure to an antiCD51 antibody; and

FIGURE 14 shows results from mouse popliteal lymph node assay.

35

EXAMPLE 1

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Generation of antigen-presenting cells**(a) Dendritic cells**

Immature dendritic cells were derived from peripheral
5 human blood cells using standard procedures as
described by Sallusto et al (1995) J. Exp. Med. 182
pp 389-400. Briefly, monocytes were cultivated in
RPMI 1640 supplemented with 2mM Glutamine, 50 µg/ml
10 Kanamycin, 1% nonessential amino acids (GibcoBRL), 10%
human AB serum and 50 ng/ml of each IL-4 (specific
activity $> 2 \times 10^6$ U/mg, PeproTech) and GM-CSF (specific
activity $> 1 \times 10^7$ U/mg, Schering-Plough) for 6 days.
Between day six and day nine of the culture non-
adherent immature dendritic cells were harvested and
15 purified by depletion of contaminating lymphocytes
with the aid of magnetic beads (Dynal) and anti-CD3
and anti-CD19 monoclonal antibodies (DAKO).

(b) Monocytes

20 Monocytes were purified from peripheral blood by
adherence of PBMC to plastic dishes for 2 hours. Non
adherent cells were removed and the adherent cells
layer washed 2 times with warm PBS. For further
25 purification, the adherent cells were detached by
incubation with 2 mM EDTA in PBS for 20 min and
contaminating lymphocytes depleted with the aid of
magnetic beads (Dynal or Miltenyi) and anti-CD3 and
anti-CD19 monoclonal antibodies (DAKO).

30

(c) Macrophages

Monocytes isolated as described above were cultured in
RPMI 1640 supplemented with 2 mM Glutamine, 50 µg/ml
35 Kanamycin, 10% human AB serum and 50 ng/ml of M-CSF
(specific activity $> 2 \times 10^6$ U/mg, Peprotech) for 6

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days.

(d) B-lymphocytes

5 B-lymphocytes were isolated from human blood according to standard procedures. Briefly, non-adherent PBMC were subjected to depletion of contaminating monocytes and T-cells with the aid of magnetic beads (Dynal or Miltenyi) and anti-CD14 and anti-CD3 monoclonal
10 antibodies (DAKO). B-cells were cultured in RPMI 1640 supplemented with 2 mM Glutamine, 50 µg/ ml Kanamycin, 1% to 10% human AB serum.

(e) CD34+ Cells

15 CD34+ cells were isolated from PBMC with the aid of anti-CD34 antibody conjugated magnetic beads (Dynal or Miltenyi). CD34+ progenitor were then cultured in RPMI 1640 supplemented with 2 mM Glutamine, 50 µg/ ml
20 Kanamycin, 1% to 10% human AB serum and the following cytokines: 100 ng/ml of GM-CSF (Schering-Plough), 50 ng/ml TNFα and 50 ng/ml SCF (Peprotech) for 12 days. As an alternative, CD34+ cells could be expanded in the above mentioned medium but supplemented with 25
25 ng/ml FLT3-L , 10U/ml TPO, SCF 20 ng/ml (Peprotech) for up to 8 weeks and then induced to differentiate to dendritic cell by culture of a further 3 days in medium supplemented with 25 ng/ml GM-CSF and 25 ng/ml IL-4."

30

EXAMPLE 2

Maturation assay

35 (a) For maturation assays 1×10^6 purified dendritic cells were incubated in duplicate wells (a) with

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100 ng/ml LPS, (b) with 100ng/ml LPS with or without prior exposure to 1×10^8 RBC, (c) with 100 ng/ml LPS with or without prior exposure to parasite lysate corresponding to 1×10^8 parasite infected RBC, (d) 100
5 ng/ml LPS with or without prior exposure to 1×10^8 intact ITO/A4 infected RBCs. Incubation with LPS (*Salmonella typhimurium*) was for a period of 48 hours.

Maturation of the dendritic cells was measured using
10 monoclonal antibodies to the following human cell surface markers: CD3 clone OKT3, HLA A,B,C clone W32/6, CD14 clone Tuk4, CD54 clone 6.5B5, CD19 clone HD37 (DAKO): CD36 clone 89 (IgG1) or clone SMQ(IgM), CD80 clone BB1, CD40 clone LOB7/6, CD86 clone BU63,
15 HLA DR clone BF-1 (Serotec), CD83 clone HB15a (Zhou et al (1995) J. Imm. 154, pp3821-3835. Staining of dendritic cells was performed as described by Zhou et al above and immunofluorescence analysed by FACScan (Becton Dickenson). All experiments were repeated at
20 least six times with dendritic cells obtained from different donors. Dead cells were excluded from analysis using Propidium Iodide. The results are shown on Figure 3. The relative increase of surface expression is expressed as the mean fluorescence
25 intensity (MFI) of matured dendritic cells over the MFI on immature dendritic cells.

The results show that dendritic cell maturation is inhibited by the direct interaction with intact
30 infected erythrocytes and is not due to the secretion of inhibitory parasite products or a toxic effect of parasite debris.

The differences in surface expression on dendritic
35 cells exposed to intact infected erythrocytes to dendritic cells alone are statistically significant for all markers with $p < 0.01$ (Student t-test).

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(b) Other antigen-presenting cells

For maturation assays 1×10^6 purified monocytes or macrophages were incubated in duplicate wells with or without LPS in the presence of medium alone, antibodies such as anti-CD36 or anti-CD51 or control antibodies, apoptotic cells or necrotic cells. Maturation was measured by the increase in surface expression of HLA-DR, CD54 and CD86 using the antibodies and flow cytometry as described above.

For maturation assays 1×10^6 purified B-cells were incubated in duplicate wells with or without LPS in the presence of medium alone, antibodies such as anti-CD36 or anti CD51 or control antibodies, apoptotic cells or necrotic cells. Activation was measured by the increase in surface expression of HLA DR (clone BF-1, Serotec), CD86 (clone BU63, Serotec) and CD40-Ligand (clone TRAP1, Pharmingen) and flow cytometry.

EXAMPLE 3**Cultivation of Plasmodium falciparum infected red blood cells**

Laboratory strains of Plasmodium falciparum were cultured in human RBC as described by Trager et al (1976) Science. 193 pp673 to 675. The cytoadherent cell lines ITO/A4 and ITO/C24 were clones isolated by manipulation from the ITO4 line, which is derived from a parasite isolate from Ituxi in Brazil. The cytoadherent parasite line Malayan Camp (MC) and the non-adherent cell line T9/96 were both adapted to in vitro culture from parasites originally isolated from Thailand.. All cultures were free from mycoplasma contamination. Infected erythrocytes were purified

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either by differential sedimentation in Plasmagel or through 65% Percoll both of which gave a yield of more than 90% infected erythrocytes. Examination of a thin film revealed that more than 90% of infected erythrocytes were viable. Parasite lysate was obtained by three rounds of freezing and thawing of mature infected RBC. Parasite pigment was prepared as described by Schwarzer et al (1994) BR. J. Haematol. 88, pp740-745. Parasite conditioned medium was the supernatant derived after culturing 1×10^8 purified infected erythrocytes in dendritic cell medium for 24 hours. All materials were from Sigma unless otherwise stated.

15 **EXAMPLE 4**

Binding of parasites to purified proteins

Binding of parasitised RBCs to purified proteins was measured as previously described by Craig et al (1997) Infect. Immun. 65, pp 4580-4585. Briefly, two microlitres of a solution of TSP (Gibco-BRL), purified CD36 or purified CD54 (ICAM-Fc) were adsorbed onto bacteriological, plastic plates. Mature erythrocytes parasitised with *P. falciparum* strains (a) ITO/A4, (c) ITO/C24, (e) MC and (g) T9/96, were suspended in binding medium and added to each dish. The erythrocytes were allowed to settle and then resuspended by gentle rotation every 10 minutes for 1 hour. Non-adherent cells were removed, the remaining cells fixed and stained with Giemsa. Adherent parasitised cells were counted by light microscopy and the number of cells bound per square millimeter were corrected to binding at 2% haematocrit and 5% parasitaemia. The results are shown in Figure 4A and confirm that like ITO/A4, ITO/C24 and MC are able to

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adhere to CD36 and TSP. However, their adherence to CD54 was much reduced. T6/96 does not adhere to CD54, CD36 or TSP.

5 **EXAMPLE 5**

Effect of parasite strains on maturation

10 A maturation assay as described in Example 2 was carried out but exposing immature dendritic cells to erythrocytes infected with (b) ITO/A4, (d) ITO/C24, (f) MC and (h) T9/96. The results are shown in Figure 4B. While parasite lines MC and ITO/C24 inhibited the maturation of dendritic cells in a similar vein to
15 clone ITO/A4, the non-adherent line T9/96 did not inhibit maturation of dendritic cells even at a ratio of infected erythrocytes to dendritic cells of 100:1.

20 **EXAMPLE 6**

Electron microscopy

Adherence of ITO/A4 infected erythrocytes but not T9/96 infected erythrocytes to dendritic cells was
25 confirmed by electron microscopy. One million purified immature dendritic cells were incubated for 2 hours and for 12 hours with 1×10^8 ITO/A4 infected RBC (a) or T9/96 infected (d) in 2 ml of dendritic cell medium, harvested and fixed with 2.5%
30 glutaraldehyde/cacodylate buffer. Cells were post fixed in osmium tetroxide, dehydrated and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate prior to examination in a Joel 1200EX electron microscope. The number of
35 adherent and infected erythrocytes and the number of phagosomes containing pigment granules was counted in

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each sample in thin sections of 100 randomly selected dendritic cells. Transmission electron micrographs are shown in Figure 5.

- 5 Note the cell processes partially enclosing infected erythrocytes (arrows in a) and the close apposition of the limiting membranes of the infected erythrocytes and dendritic cells particularly at the knobs (b, arrowhead). Within dendritic cell cytoplasm are
10 phagosomes containing characteristic pigment granules (c, arrows). N - dendritic cell nucleus, P - infected erythrocyte. Bars are 2 μ m (a and d), 200 μ m (b), 500 μ m (c).
- 15 ITO/A4 infected erythrocytes were observed to be in intimate contact with immature dendritic cells with cytoplasmic processes partially enclosing the parasites (Fig. 5a). The plasmalemma of the infected erythrocytes was in close apposition to the limiting
20 membrane of the dendritic cell particularly at the site of knobs (Fig. 5b). A similar apposition between parasitised erythrocytes and host cells is seen between infected red blood cells and endothelial cells (Berendt et al (1994) *Parasitology* 108 Suppl. 519-28).
- 25 In contrast, only a few infected erythrocytes of the T9/96 strain were associated with the dendritic cells (Fig. 5d). When quantified, ten times more ITO/A4 infected erythrocytes were found adherent to dendritic
30 cells than T9/96 infected erythrocytes in 100 thin sections of dendritic cells. Furthermore, ingestion of intact ITO/A4 infected erythrocytes by dendritic cells was not observed during this time. Nevertheless, phagocytosis of parasite debris as revealed by the
35 number of phagosomes containing pigment granules (Fig. 5c) was similar for dendritic cells incubated with ITO/A4 or with T9/96.

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EXAMPLE 7**T-cell proliferation assays**

- 5 Total-T-cells (allogeneic MLR) or CD4+ T cells
 (primary T-cell responses) were purified using a
 Collect column (TCS). For the allogeneic MLR,
 dendritic cells were added in increasing numbers (156
10 to 10,000) to 1×10^5 T-cells in triplicate and
 incubated for 5 days. T-cells were pulsed with 0.5 μ Ci
 3 H-thymidine/well for the last 18 hours of the
 culture. For primary T-cell responses, 1×10^6
15 dendritic cells were incubated with medium alone or
 with 1×10^8 infected erythrocytes for 18 h and then
 pulsed with 10 μ g/ml parasite-lysate or with 30 μ g/ml
 keyhole limpet haemocyanin, respectively. The
 dendritic cells were purified by sedimentation through
 Lymphoprep™ and 1×10^5 dendritic cells were culterd
20 with 1.5×10^6 CD4+ T-cells from the same donor. From
 day 4 to day 6 of culture, 50 μ l aliquots were taken
 in triplicate and pulsed with 0.5 μ Ci
 3 H-thymidine/well for 8 hours.
 (see Plebanski et al (1992) Immunol. 75 86-90). The
 results are shown in Figure 6.
- 25 Dendritic cells exposed to intact infected
 erythrocytes are poor stimulators of T-cell
 proliferation. Allogeneic T-cell proliferation (a)
 induced by immature dendritic cells (■), LPS-matured
30 dendritic cells (□) and dendritic cells co-cultivated
 with intact ITO/A4 infected erythrocytes (▼) prior to
 maturation. Primary CD4+ T-cell responses to
 parasite-lysate (b) and to keyhole limpet haemocyanin
 (c) induced by LPS-matured autologous dendritic cells
35 (□,○) and autologous dendritic cells co-cultivated
 with intact ITO/A4 infected erythrocytes (■,) prior

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to maturation. Data from one out of three independent experiments are shown.

5 Dendritic cells matured after incubation with
uninfected RBC, a crude pigment preparation or a
lysate of infected erythrocytes induced a similar
degree of T-cell proliferation in a mixed leukocyte,
reaction to that induced by control mature dendritic
cells (data not shown).
10
However, dendritic cells incubated with LPS after
exposure to intact infected erythrocytes from the
parasite line ITO/A4 were strikingly less efficient in
their induction of T-cell proliferation compared with
15 the T-cell proliferation induced by mature dendritic
cells (Fig 6a). Furthermore, dendritic cells exposed
to intact infected erythrocytes before maturation with
LPS did not induce primary CD4+ T-cell responses to
lysate of infected erythrocytes or to keyhole limpet
20 haemocyanin (Plebanski et al) (Fig 6, b,c).

It is concluded that the maturation of dendritic
cells and their subsequent ability to activate
T-cells is profoundly inhibited by their interaction
25 with intact infected erythrocytes. Non-adherent
parasite lines, parasite debris and crude pigment do
not modulate dendritic cell function in this way.
These studies provide one explanation for the
clinical and experimental evidence of immune
30 dysregulation during malaria infection such as the
impairment of the delayed-type hypersensitivity
response to recall antigens and the antibody response
to vaccines.

35 **EXAMPLE 8**

Maturation assay with monoclonal antibody

- 35 -

A maturation assay was carried out as described in Example 2 except that instead of infected erythrocytes the immature dendritic cells were exposed to monoclonal antibodies to CD36, CD51 or both prior to immune stimulation with LPS. Specifically, 1×10^6 purified dendritic cells were incubated in duplicate wells without or with either 25 μ g irrelevant IgM antibody, 25 μ g irrelevant IgG1 antibody, 25 μ g antiCD36 antibody, 25 μ g antiCD51 antibody or a combination thereof for at least 3 hours. Thereafter, dendritic cells were matured with 100 ng/ml LPS (*Salmonella typhimurium*) for 48 hours or left untreated as a control. The monodonal antibodies tested were CD36 clone SMQ (Immunocontact) and clone 89 (Serotec), CD51 clone 13C2 (Immunocontact), IgM isotope control clone MOPC, IgG isotope control clone MOPC (Sigma). The results of two experiments are shown in Figures 7 and 8 respectively. As will be apparent both CD36 and CD51 antibodies have the effect of inhibiting dendritic cell maturation in a similar manner to infected erythrocytes.

EXAMPLE 9

Maturation assay with apoptotic cells

A maturation assay was carried out as described in Example 2 except that instead of infected erythrocytes the immature dendritic cells were exposed to apoptotic cells prior to immune stimulation with LPS. Apoptotic or necrotic cells were derived from purified autologous dendritic cells, monocytes or neutrophils. Specifically for maturation assays in the presence of apoptotic bodies 1×10^6 purified dendritic cells were incubated in

- 36 -

duplicate wells without or with either 2×10^6 autologous apoptotic or necrotic cells for 12 hours. Maturation was induced by the addition of LPS or TNF α as stated above. Apoptosis was induced by radiation with a calibrated UV lamp at a dose of 2500 mJ/cm² and evaluated by staining with FITC-AnnexinV/Propidium Iodide according to manufacturers recommendations (Roche Diagnostics) 3 hours and 12 hours after UV radiation. Necrosis was induced by at least three cycles of rapid freezing at -70°C and thawing at 37°C. Thereafter, more than 90% of cells were permeable for trypan blue. The results are shown in Figure 9 as follows:

Apoptotic cells but not necrotic cells inhibit the maturation of dendritic cells. (a) Immature dendritic cells were left untreated, matured with LPS or exposed autologous apoptotic or necrotic dendritic cells prior to maturation with LPS and subsequently stained with antibodies directed against surface marker and analysed by FACScan as indicated. (b) Dead Cells and especially apoptotic cells were efficiently excluded from analysis by gating on forward scatter and exclusion of cells positive for Propidium Iodide. (c) Proliferation of allogeneic T-cells stimulated by increasing numbers of immature dendritic cells (◆), LPS-matured cells (▲) or dendritic cells exposed to apoptotic dendritic cells and matured with LPS (■).

The results of a further experiment with apoptotic neutrophils shown in Figure 10.

EXAMPLE 10

Antigen specific T-cell responses

The proliferative response of the CD4+ T-cell clone

- 37 -

TB-2 for the peptide 144-163 of the human acetylcholine receptor (Nagvekar N et al, J. Clin invest, 1998 101 (10) pp 2268-77) was analysed. T-cell proliferation was measured as described in Example 7.

- 5 For antigen-specific T-cell responses, 1×10^6 dendritic cells were incubated with medium alone or with antibodies as indicated and then pulsed for 6 h with 0.025 mM AChR α : 3-181 polypeptide before or 1 mM AChR α :144-163 peptide after maturation with LPS.
- 10 For antigen specific T-cell responses of the clone TB-2 increasing numbers of MHC class II matched dendritic cells were incubated with 3×10^4 T-cells for 72 h. Proliferation was measured in all assays by adding 0.5 mCi ^3H -thymidine/well for the last 8 hours
- 15 of the culture.

- The results are shown in Figure 11 (c) and (d). Exposure of dendritic cells to antiCD36, antiCD51 or both antibodies abolished their ability to induce
- 20 proliferation in the T-cell clone as compared to dendritic cells exposed to irrelevant antibodies (Fig 2c, d). The proliferation of the T-cell clone remained low even when the modulated dendritic cells were exogenously loaded with peptide thus excluding a
- 25 defect in antigen-uptake or antigen-processing due to the presence of antibodies.

EXAMPLE 11

30 Cytokine production by dendritic cells

- Secretion of the cytokines TNF alpha, IL12 p70 and IL10 was measured in supernatants of dendritic cells treated with either anti-CD36 antibody or apoptotic
- 35 cells or in the respective controls before or after maturation with LPS for 24 hours using commercially

- 38 -

available ELISA kits. These kits can be obtained from R and D Systems, Europe Ltd. 4-10, The Quadrant, Barton Lane, Abingdon, Oxford, OX143 YS and BD Pharmingen, 10975, Torreyana Road, San Diego, CA 92121, USA.

Maturation with LPS induced secretion of TNF- α by dendritic cells irrespective whether they were exposed to anti-CD36 antibodies or apoptotic cells although the concentration of TNF- α was consistently slightly lower than in the respective controls. IL12 p70 was secreted by control dendritic cells matured with LPS whereas IL10 was secreted by dendritic cells exposed to anti-CD36 antibodies or apoptotic cells. Of note, the absolute amount of IL10 varied considerably between dendritic cells treated with anti-CD36 and dendritic cells exposed to apoptotic cells in response to LPS. It is possible that intact cells bind to more than one receptor thus modifying the cytokine secretion induced by CD36 alone. However, we investigated whether secretion of IL10 had a role in inhibition of dendritic cell maturation due to ligation of CD36 by maturing dendritic cells exposed anti-CD36 antibodies or to apoptotic cells in the presence of blocking anti-IL10 antibodies. The inhibition of dendritic cell maturation was not reversed (data not shown) and is therefore independent of the secretion of IL10.

EXAMPLE 12

Modulation of mouse dendritic cell maturation and function by antiCD51 antibodies in vitro and in vivo

Rationale

Since human monocyte derived dendritic cells can be modulated in their maturation and function by a variety

- 39 -

of agents including antibodies binding to CD36 and or CD51, in this study we began to investigate whether a similar phenomenon could be observed in mouse dendritic cells.

5

Methods

Generation of bone-marrow derived dendritic cells: Bone marrow from male Balb/c (H-2^k) mice was harvested and total cells were cultured in RPMI supplemented with 2 mM glutamine, 50 mg Kanamycin, 10 % FCS, 10 ng/ml each murine recombinant GM-CSF and IL-4. On day two of culture half the medium was replaced with fresh medium supplemented with cytokines and on day four of culture non-adherent cells were harvested.

15

In vitro maturation of bone-marrow derived mouse dendritic cells: One million of bone marrow derived dendritic cells (approximately 50% total cells) in duplicate were exposed to either medium alone, 25 mg isotype control antibody or 25 mg antiCD51 antibody for 8 hours. Cells were subsequently exposed to 100 ng LPS for 48 hours or left alone as a control. Maturation of dendritic cells was analysed by double staining with FITC conjugated antibodies against CD11c and PE-conjugated antibodies directed against either CD40, CD54, CD86 or I-A and subsequent FACScan analysis. Analysis was performed on CD11c-FITC positive cells.

25

Popliteal lymph node assay: Dendritic cells were exposed to medium alone or to antiCD51 antibodies and then matured with LPS as described above. The cells were then harvested and washed four times in PBS in order to remove LPS. Cells were resuspended in 10% FCS/PBS at a concentration of 6×10^5 total cells/20 ml. Groups of six male C3H3/HE (H-2^d) mice were injected with 20 ml of PBS into the right footpad and LPS matured dendritic cells into the left footpad, with 20 ml

30

35

- 40 -

of PBS into the right footpad and dendritic cells exposed to antiCD51 antibody prior to LPS maturation into the left footpad or with 20 ml of PBS into the right and the left footpad. After one week mice were sacrificed and the popliteal lymphnodes were removed. The weight of the left and the right lymphnode of each mouse in all three groups were determined and the ratio of the weight of the left lymphnode to the weight of the right lymphnode was calculated. The mean and SE of the ratio was determined for each group.

Results

Dendritic cells matured with LPS (DC LPS) increased the surface expression of the molecules CD40, CD54 and CD86 as compared to immature dendritic cells (DC). However, when dendritic cells were treated with antiCD51 antibodies prior to exposure to LPS (DC CD51 lps), the dendritic cells failed to mature and the expression of surface molecules remained at the level of immature dendritic cells (DC). The antibody itself had no effect on dendritic cell maturation (DC CD51). The results are shown in Figure 13.

When dendritic cells matured with LPS were injected into the footpad of allogeneic C3H/HE mice, they induced a strong allogeneic T-cell response as measured by the increase in weight of the popliteal lymphnode of the right footpad compared to the popliteal lymphnode of the left footpad. By contrast, the increase in weight of the right popliteal lymphnode over that of the left popliteal lymphnode was markedly reduced when dendritic cells were exposed to antiCD51 antibody prior to maturation with LPS. However, the overall increase in weight was still above that of popliteal lymphnodes of mice that had been injected with PBS in both footpads. The results are shown in Figure 14.

EXAMPLE 13**Modulation of human dendritic cells *ex vivo***

5 Agonists of CD36, CD51, thrombospondin receptors or
β-integrin may be used to modulate human immune
response in patients with unwanted and/or harmful
allo- or auto-immune responses. For such a method of
10 treatment immature human dendritic cells which are
defined and identifiable as described herein and with
the ability to phagocytose are derived from
preparations of human peripheral blood.
Specifically, the dendritic cells are derived from
CD34+ stem cells or monocytes isolated from human
15 peripheral blood by the method described in Example
1. To the preparation of cells suspended in
appropriate medium, for example RPMI as defined
herein, an agonist of C36, CD51, thrombospondin
receptor or β-integrin is added. The relative
20 concentration of agonist to cells is adjusted
depending on the nature of the agonist used. For
example, if the agonist is a monoclonal antibody,
about 25 µg antibody to about 10⁶ dendritic cells is
appropriate at a concentration of 25 µg Ml⁻¹. The
25 cells are treated for between 3 and 24 hours.

Depending on the particular application cells are
returned to the individual from which they were
originally derived or administered to another
individual. Adminitration may be by intravenous
30 infusion, by inhalation or by sub-cutaneous or
intramuscular injection.

Administration of dendritic cells to a human
following the method described above will give rise
to a generalized immune suppressive effect which will
35 be useful, in a number of situations, for example in
the prevention of rejection of allografts and

- 42 -

xenografts or for treatment of disease suspected of having an auto-immune basis but for which the auto-antigen is not known.

5 However, the method described above may be modified to produce dendritic cells which are tolerant to a specific antigen. In this case the dendritic cell preparation is exposed to an antigen against which tolerance is to be induced as well as to the CD36, CD51, thrombospondrin receptor or β -
10 integrin agonist. The cells may be exposed to antigenic material, before, after or simultaneously with the aforesaid agonist molecule. The antigenic material may be linked to, fused to or otherwise associated with said agonist molecule. Exposure to
15 the antigenic material is for about 6 to about 24 hours with or without an immune stimulant, then the cells are reintroduced to the patient as described above.

20 The ability to induce tolerance to a specific antigen, for example an allo, xeno or auto-antigen allows a great many therapeutic applications. For example tolerance can be induced in respect of the following antigens:

25 to major or minor histocompatibility antigens of a recipient of a bone marrow transplant (to modulate graft versus host disease in bone marrow transplantation in dendritic cells from the bone marrow donor) or

30 to donor major or minor histocompatibility antigens in dendritic cells of recipients of solid organ transplant or

35 to antigens to which there is pathological immune response causing auto-immune diseases for example: components of autologous red blood cells to modulate

- 43 -

- the immune response in patients with auto-immune hemolytic anaemia
- components of autologous platelets in patients with auto-immune thrombocytopenia
- 5 components of beta islet cells of the pancreas in patients with insulin dependent diabetes mellitus
- components of other endocrine organs in patients with other organ specific auto-immune diseases
- 10 components of the acetylcholine receptor in patients with myasthenia gravis
- other antigens or apoptotic cells containing antigens causing harmful or pathological immune responses in other auto-immune diseases
- 15 to antigens to which there is pathological immune response causing atopic or allergic diseases, for example antigens eliciting an immune response in hay fever, asthma, eczema or coeliac disease
- 20 to antigens to which there is pathological immune response that may be defined in other diseases for example in non-organ specific immune diseases (systemic lupus erythematosus or rheumatoid arthritis) or other immune mediated arthritis or
- 25 other connective tissue diseases in inflammatory bowel disease in auto-immune hepatitis in multiple sclerosis or in other auto-immune disease
- 30 to allo-antigens to which there is a harmful or pathological immune response for example components of red blood cells in haemolytic disease of the newborn or in previously transfused patients
- 35 components of platelets for example in neo-natal allo immune thrombocytopenia or in conditions where there is an allo-immune response to transfused platelets

- 44 -

other blood products or substitutes for example
Factor VIII in haemophilia patients
other synthesized or manufactured or naturally
occurring products or substances

5

Although the treatment of humans with dendritic
cells is described in this example it will be
appreciated that other antigen-presenting cells, such
as macrophages, monocytes or β -lymphocytes could be
used for treatment with an agonist of CD36, CD51,
thrombospondin receptor or β -integrin and optionally
an antigenic material. Specific tolerance can be
introduced in such cells for use in any of the
applications listed above.

15

Example 14

Preparation of phosphatidylserine liposome compositions and their therapeutic uses.

Liposomes containing phosphatidylserine or other
negatively charged phospholipids, with or without
additional targeting molecules, induce general immune
unresponsiveness. Liposomes encapsulating antigens
and phospholipids with or without additional
targeting molecules induce antigen specific immune
unresponsiveness.

Liposomes are prepared as described by Coradini
et al, Anticancer Research 1998 18 177-182. In brief
clean glass tubes are coated with 2 micromolar of
mixtures of phosphatidylcholine and
phosphatidylserine, other negatively charged
phospholipids or other phospholipids including
cholesterol and/or cholesterol ester dissolved in
chloroform. The solvent is evaporated under nitrogen
gas and the tubes incubated in a vacuum for 45
minutes. Sterilised phosphate buffered saline

- 45 -

(unmodified liposomes) or containing the antigen(s) to which unresponsiveness will be induced with or without molecules allowing targeting of the liposomes to CD36 and/or CD51 and/or beta-integrins and/or other receptors of apoptotic cells or other molecules expressed on the surface of antigen presenting cells, is added to the lipid shell. Suitable targeting molecules are monoclonal antibodies to the respective receptors or fragments of the P. falciparum erythrocyte membrane protein-1 that bound to CD36 and/or thrombospondin. The tubes are shaken at high speed for 5 minutes and separated from free fatty acid by ultracentrifugation at 100,000g for 60 minutes. Targeting molecules (see above) may be covalently or non-covalently attached to the surface of liposomes. The liposomes are filtered through a 0.22 micrometer filter. Encapsulation of antigens and targeting molecules can also be achieved by freeze-thawing or dehydration/rehydration or by reverse phase evaporation (Monnard PA et al, Biochem. Biophys. Acta 1997 1329 39-50) or by other published methods of preparing liposomes.

Liposomes prepared as described above would be added to 1×10^6 isolated immature dendritic cells or to other antigen presenting cells at a concentration of 25 micrograms per ml. The maturation and function of the dendritic cells or other antigen presenting cells is assessed as previously described. The liposomes containing phosphatidylserine (with or without targeting molecules) is used to treat dendritic cells or other antigen presenting cells ex vivo or for systemic treatment.

APPENDIX 1

DI is distributor
SD is standard designation

Other MABs are

OKM5	Ortho Pharmaceutical Corporation
OKM8	1001 US Highway 202
	P.O. Box 250
	Raritan, N.J.

cd 36 in HDB-DI HDB-NONDI

<http://www.atcc.org/cgi-bin/Sfgat...abase=local%2FHDB-NONDI&text=cd36>

Your query was:

cd36

The selected databases contain 18 documents matching your query:

- 1: 1013396 RE 1.CE>platelet 1.SN>CD36 1.a.CC>differentia
- 2: 1003558 RE 1.CE>platelet 1.U>cell membrane 1.SN>CD36
- 3: 1018253 RE 1.SN>CD36 1.a.CC>differentiation 1.b.CC>gly
- 4: 1020319 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
- 5: 1013397 RE 1.CE>platelet 1.SN>CD36 1.a.CC>differentia
- 6: 1020540 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
- 7: 1017636 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
- 8: 1022016 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
- 9: 1019865 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
- 10: 1024459 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
- 11: 1019119 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36 1.MW
- 12: 1009963 RE 1.G>Homo sapiens 1.CN>human 1.CE>monocyte
- 13: 1016854 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet
- 14: 1023242 RE 1.G>Homo sapiens 1.CN>human 1.U>cell membr
- 15: 22825 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet
- 16: 1012440 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
- 17: 1012380 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
- 18: 1003358 RE 1.SN>ACTH 1.FS>N-terminal region 1.a.CC>ho

- 48 -

013396 RE 1.CE>platelet 1.SN>CD36 1.a.CC>differentia

1013396 RE 1.CE>platelet 1.SN>CD36 1.a.CC>differentiation

AN 1013396

DI P>Medica

DI 2382 Camino Vida Roble, Suite I

DI Carlsbad, CA 92009 USA

DI 1-619-438-1886

DE C>CLB/703 ;developer

DE P>MON1118 ;distributor

PD ;IgG1

RE 1.CE>platelet 1.SN>CD36 1.a.CC>differentiation

RE 2.CE>monocyte 2.SN>CD36 2.a.CC>differentiation

RE 3.CE>macrophage 3.SN>CD36 3.a.CC>differentiation

RE 4.CE>platelet 4.SN>CD36 4.a.CC>differentiation

AP ;frozen section

SD MON1118

LD USA BAL

EI DA>9303

CI ;catalog

SN Synonym>CD36 1013396 SN Synonym>CD36

_003558 RE 1.CE>platelet 1.U>cell membrane 1.SN>CD36

1003558 RE 1.CE>platelet 1.U>cell membrane 1.SN>CD36

AN 1003558

DI P>Biodesign International

DI 105 York Street

DI Kennebunkport, ME 04043 USA

DI 1-207-985-1944

DE P>N42540M ;distributor

PD ;IgG1

RE 1.CE>platelet 1.U>cell membrane 1.SN>CD36

RE 1.a.CC>differentiation 1.b.CC>protein

RE 1.c.CC>blood coagulation factor

AV ;purified

SD N42540M

LD USA BAL

EI DA>9002 CV>9007

CI ;catalog

SN Synonym>CD36 1003558 SN Synonym>CD36

018253 RE 1.SN>CD36 1.a.CC>differentiation 1.b.CC>gl

1018253 RE 1.SN>CD36 1.a.CC>differentiation 1.b.CC>glycoprotein

AN 1018253

DI P>BioGenex Laboratories

DI 4600 Norris Canyon Road

DI San Ramon, CA 94583 USA

DI 1-510-275-0550

DI 1-800-421-4149 (toll free USA)

DE P>1E8 ;distributor

DO G>Mus musculus CN>mouse

RE 1.SN>CD36 1.a.CC>differentiation 1.b.CC>glycoprotein

AV ;purified

SD 1E8

LD USA JMJ

EI DA>9602

CI ;catalog

SN Synonym>CD36 1018253 SN Synonym>CD36

- 51 -

020319 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

1020319 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

AN 1020319

DI P>Harlan Bioproducts for Science, Inc.

DI P.O. Box 29176

DI Indianapolis, IN 46229-0176

DI 1-317-894-7536

DI 1-800-9-SCIENCE

DE C>89 ;distributor

DE P>MCA1214 ;distributor

DO G>Mus musculus CN>mouse

PD ;IgG2b

RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

RE 1.a.CC>differentiation

AP ;flow cytometry ;Western blot

AV ;purified

SD 89

SD MCA1214

LD USA CLB

EI DA>9702

CI ;catalog

SN Synonym>CD36 1020319 SN Synonym>CD36

013397 RE 1.CE>platelet 1.SN>CD36 1.a.CC>differentia

1013397 RE 1.CE>platelet 1.SN>CD36 1.a.CC>differentiation

AN 1013397

DI P>Caltag Laboratories

DI 1849 Bayshore Blvd. #200

DI Burlingame, CA 94010

DI 1-650-652-0468

DI 1-800-874-4007

DI 2.P>Medica

DI 2382 Camino Vida Roble, Suite I

DI Carlsbad, CA 92009 USA

DI 1-619-438-1886

DE C>VM58 ;developer

DE P>MON1143 ;distributor

DE P>VM58 ;distributor

DE 2.P>MON1143 ;distributor

PD ;IgG1

RE 1.CE>platelet 1.SN>CD36 1.a.CC>differentiation

RE 2.CE>monocyte 2.SN>CD36 2.a.CC>differentiation

RE 3.CE>macrophage 3.SN>CD36 3.a.CC>differentiation

RE 4.CE>platelet 4.SN>CD36 4.a.CC>differentiation

AP ;frozen section

SD VM58

LD USA BAL

EI DA>9303

CI ;catalog

SN \$ynonym>CD36 1013397 SN Synonym>CD36

- 53 -

_020540 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

1020540 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

AN 1020540

DI P>Harlan Bioproducts for Science, Inc.

DI P.O. Box 29176

DI Indianapolis, IN 46229-0176

DI 1-317-894-7536

DI 1-800-9-SCIENCE

DE P>89 ;developer

DE P>MCA1214 ;distributor

DO G>Mus musculus CN>mouse

PD ;IgG2b

RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

RE 1.a.CC>differentiation

AP ;flow cytometry ;Western blot

AV ;purified

SD 89

SD MCA1214

LD USA CLB

EI DA>9702

CI ;catalog

SN Synonym>CD36 1020540 SN Synonym>CD36

17636 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

1017636 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

AN 1017636

DI P>Novocastra Laboratories Ltd.

DI 24 Claremont Place

DI Newcastle upon Tyne NE2 4AA, UK

DI 44-0191 222 8550

DE P>NCL-CD36 ;distributor

DE P>SMO ;distributor

DO G>Mus musculus CN>mouse

AS ;immunohistochemical staining

RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

RE 1.a.CC>differentiation

AP ;frozen section

AV ;ascites

SD NCLCD36

SD SMO

LD USA BAL

EI DA>9904

CI ;catalog

SN Synonym>CD36 1017636 SN Synonym>CD36

022016 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

1022016 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

AN 1022016

DI P>O.E.M. Concepts, Inc.

DI 1889 Route 9, Bldg. 25, Unit 96

DI Toms River, NJ 08755 USA

DI 1-732-341-3570

DE C>289-10930 ;distributor

DE P>M2-L69 ;distributor

DO G>Mus musculus CN>mouse

RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

RE 1.a.CC>differentiation

AP ;cell surface marker

AV ;purified

SD 28910930

SD M2L69

LD USA EJK

EI DA>9712

CI ;catalog

SN Synonym>CD36 1022016 SN Synonym>CD36

019865 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

1019865 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

AN 1019865

DI P>Upstate Biotechnology, Inc.

DI 199 Saranac Avenue

DI Lake Placid, NY 12946 USA

DI 1-617-890-8845

DI 1-800-233-3991 (toll free USA) (sales)

DE P>05-287 ;distributor

DO G>Mus musculus CN>mouse S>BALB/c O>spleen

PD ;IgM

RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

RE 1.a.CC>differentiation 1.b.CC>protein

AP ;immunocytochemistry

AV ;ascites

SD 05287

LD USA JMJ

EI DA>9611

CI ;catalog

SN Synonym>CD36 1019865 SN Synonym>CD36

- 57 -

016854 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet

1009963 RE 1.G>Homo sapiens 1.CN>human 1.CE>monocyte

1009963 RE 1.G>Homo sapiens 1.CN>human 1.CE>monocyte 1.SN>CD36

AN 1009963

DI P>Harlan Bioproducts for Science, Inc.

DI P.O. Box 29176

DI Indianapolis, IN 46229-0176

DI 1-317-894-7536

DI 1-800-9-SCIENCE

DI 2.P>Immunotech S.A.

DI Departement commercial

DI Luminy Case 915

DI 13288 Marseille Cedex 9, France

DI 33-91-41-41-38

DI 430246 F IMMTECH

DE C>Fa6-152 ;developer

DE P>MCA 682 ;distributor

DE 2.P>0765 ;distributor

DE 2.P>0766 ;distributor

DE 2.P>FA6.152 ;distributor

DO G>Mus musculus CN>mouse

PD ;IgG1

RE 1.G>Homo sapiens 1.CN>human 1.CE>monocyte 1.SN>CD36

RE 1.a.CC>differentiation

RE 2.G>Homo sapiens 2.CN>human 2.CE>macrophage 2.SN>CD36

RE 2.a.CC>differentiation

RE 3.G>Homo sapiens 3.CN>human 3.CE>platelet 3.SN>CD36

RE 3.a.CC>differentiation

AV ;purified ;2.fluorescein conjugate ;2.purified

SD 0765

SD 0766

SD FA6152

SD MCA682

LD USA BAL

EI DA>9103 CV>9104

CI ;catalog

SN Synonym>CD36 1009963 SN Synonym>CD36

SUBSTITUTE SHEET (RULE 26)

- 58 -

_023242 RE 1.G>Homo sapiens 1.CN>human 1.U>cell membr

1023242 RE 1.G>Homo sapiens 1.CN>human 1.U>cell membrane 1.SN>CD36

AN 1023242

SO Exp Cell Res 1992;198:85-92

SO J Exp Med 1990;171:1883-92

DI P>Lab Vision-NeoMarkers

DI 47770 Westinghouse Drive

DI Fremont, CA 94539 USA

DI 1-800-828-1628

DE C>1A7 ;distributor

DE P>MS-466-P ;distributor

IM G>Homo sapiens CN>human CE>platelet SN>CD36 a.CC>differentiation

IM b.CC>glycoprotein c.CC>receptor

DO G>Mus musculus CN>mouse

PD ;IgG2b ;kappa

RE 1.G>Homo sapiens 1.CN>human 1.U>cell membrane 1.SN>CD36

RE 1.MW>88 kD 1.a.CC>differentiation 1.b.CC>glycoprotein

RE 1.c.CC>receptor

AP ;flow cytometry ;immunofluorescence ;immunoprecipitation

AP ;Western blot ;immunohistology ;gold labelling

AB platelet GPIIb, platelet glycoprotein IIIb, and OKM5-antigen.

SD 1A7

SD MS466P

LD USA MCM

EI DA>9806

CI ;catalog

SN Synonym>CD36 1023242 SN Synonym>CD36

?825 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet

22825 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet

AN 22825

AU Kemshead J

AD Imperial Cancer Research Technology;

AD Sardinia House;

AD Sardinia Street;

AD London WC2A 3NL;

AD UK;

AD Tel 01 242 1136;

AD TELEX 265107 TCRFG;

AD FAX 01 831 4991

SO Br J Haematol 1984;57:621

DE P>M148 ;developer

IM G>Homo sapiens 1.CN>human PA>medulloblastoma a.CC>neoplasm

PD ;IgG1

RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet

RE 1.U>cell surface 1.SN>CD36 1.MW>110-130 kD

RE 1.a.CC>differentiation

RE 2.G>Homo sapiens 2.CN>human 2.PA>medulloblastoma

RE 2.a.CC>neoplasm

RE 3.G>Homo sapiens 3.CN>human 3.PA>neuroblastoma

RE 3.a.CC>neoplasm

RE 4.G>Homo sapiens 4.CN>human 4.PA>rhabdomyosarcoma

RE 4.a.CC>neoplasm

AP ;immunofluorescence ;immunoprecipitation

AB in vivo imaging and therapy

SD M148

LD EUR BD FI>EUR0003951 EUR901.TXT

EI DA>8901 CV>8904

CI ;catalog

SN Synonym>CD36 22825 SN Synonym>CD36

1012380 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

1012380 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

AN 1012380
DI P>Biodesign International
DI 105 York Street
DI Kennebunkport, ME 04043 USA
DI 1-207-985-1944
DI 2.P>Harlan Bioproducts for Science, Inc.
DI P.O. Box 29176
DI Indianapolis, IN 46229-0176
DI 1-317-894-7536
DI 1-800-9-SCIENCE
DI 3.P>Lampire Biological Laboratories
DI P.O. Box 270
DI Pipersville, PA 18947 USA
DI 1-215-795-2838
DI 4.P>Sigma Chemical Company
DI P.O. Box 14508
DI St. Louis, MO 63178 9916 USA
DI 1-800-325-3010 (toll free USA)
DI 1-314-771-5750
DE C>SMO ;developer
DE P>P54168M ;distributor
DE P>SMO ;distributor
DE 2.P>MCA-722F ;discontinued designation
DE 2.P>MCA722 ;distributor
DE 2.P>SMO ;distributor
DE 3.P>LBL 268 ;distributor
DE 3.P>SMO ;distributor
DE 4.P>C 4679 ;distributor
DE 4.P>F5898 ;distributor
DE 4.P>P9312 ;distributor
DE 4.P>R6395 ;distributor
DE 4.P>SMO ;distributor
DO G>Mus musculus CN>mouse
PD ;IgM
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
RE 1.a.CC>differentiation
AV ;purified ;2.purified ;4.fluorescein conjugate
AV ;4.phycoerythrin conjugate
SD C4679
SD F5898
SD LBL268
SD MCA722
SD MCA722F
SD P54168M
SD P9312
SD R6395
SD SMO
SD SMO
LD USA BAL
EI DA>9803 CV>9111
CI ;catalog
SN Synonym>CD36 1012380 SN Synonym>CD36

- 61 -

1003558 RE 1.CE>platelet 1.U>cell membrane 1.SN>CD36

1003558 RE 1.CE>platelet 1.U>cell membrane 1.SN>CD36

AN 1003558

DI P>Biodesign International

DI 105 York Street

DI Kennebunkport, ME 04043 USA

DI 1-207-985-1944

DE P>N42540M ;distributor

PD ;IgG1

RE 1.CE>platelet 1.U>cell membrane 1.SN>CD36

RE 1.a.CC>differentiation 1.b.CC>protein

RE 1.c.CC>blood coagulation factor

AV ;purified

SD N42540M

LD USA BAL

EI DA>9002 CV>9007

CI ;catalog

SN Synonym>CD36 1003558 SN Synonym>CD36

- 62 -

1024459 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

1024459 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

DI P>Biogenesis Ltd.

DI 7 New Fields

DI Stinsford Road

DI Poole BH17 7NF, England

DI UK

DI 44-1202 660006

DE C>SM-phi IgM ;distributor

DE P>2125-3607 ;distributor

DO G>Mus musculus CN>mouse

PD ;Ig

RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

RE 1.a.CC>differentiation

AP ;immunofluorescence

AV ;fluorescein conjugate

AB CD36 is also known as platelet GPIV, GPIV, platelet GPIIIb, GPIIIb, platelet

AB glycoprotein IV, and FAT (rat).

SD 21253607

SD SMPHIIGM

LD USA MCM

EI DA>9811

CI ;catalog

SN.Synonym>CD36 1024459 SN.Synonym>CD36

- 63 -

1016854 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet

1016854 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet

AN 1016854

DI P>PharMingen

DI 10975 Torreyana Road

DI San Diego, CA 92121 USA

DI 1-619-677-7737

DI 1-800-848-6227 (toll free USA)

DE P>CB38 ;distributor

DO G>Mus musculus CN>mouse S>BALB/c

PD ;IgM ;kappa

RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet

RE 1.U>cell membrane 1.SN>CD36 1.MW>88 kD

RE 1.a.CC>differentiation 1.b.CC>glycoprotein

AP ;flow cytometry ;immunoprecipitation

AV ;fluorescein conjugate ;purified

SD CB38

LD USA JMJ

EI DA>9504

CI ;catalog

SN Synonym>CD36 1016854 SN Synonym>CD36

- 64 -

1012440 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

1012440 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36

AN 1012440
DI P>BioSource International
DI 820 Flynn Roa
DI Camarillo, CA 93012 USA
DI 1-800-242-0607(toll free USA)
DI 1-805-987-0086
DI 2.P>Cymbus Bioscience Limited
DI 2 Venture Road
DI Chilworth Research Center
DI Southampton, Hampshire SO1 7NS UK
DI 44-703-767178
DI 3.P>Roche Molecular Biochemicals
DI formerly Boehringer Mannheim GmbH
DI Sandhofer Strasse 116
DI D-68305 Mannheim Germany
DI 49-621-759 8577
DE C>SMO ;developer
DE P>AHS3601 ;distributor
DE P>AHS3608 ;distributor
DE P>CS-CD36-FI ;discontinued designation
DE P>CS-CD36-UN ;discontinued designation
DE P>SMO ;distributor
DE 2.P>CBL 168 ;distributor
DE 2.P>SMO ;distributor
DE 3.P>1441 230 ;discontinued designation
DE 3.P>1441 264 ;distributor
DE 3.P>SMO ;distributor
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36
RE 1.a.CC>differentiation
AV ;fluorescein conjugate ;3.purified
SD 1441230
SD 1441264
SD AHS3601
SD AHS3608
SD CBL168
SD CSCD36FI
SD CSCD36UN
SD SMO
LD USA BAL
EI DA>9709 CV>9111
CI ;catalog
SN Synonym>CD36 1012440 SN Synonym>CD36

- 65 -

1003358 RE 1.SN>ACTH 1.FS>N-terminal region 1.a.CC>ho

1003358 RE 1.SN>ACTH 1.FS>N-terminal region 1.a.CC>hormone

AN 1003358

DI P>Biodesign International

DI 105 York Street

DI Kennebunkport, ME 04043 USA

DI 1-207-985-1944

DI 2.P>Cymbus Bioscience Limited

DI 2 Venture Road

DI Chilworth Research Center

DI Southampton, Hampshire SO1 7NS UK

DI 44-703-767178

DE C>58 ;developer

DE P>E54008M ;distributor

RE 1.SN>ACTH 1.FS>N-terminal region 1.a.CC>hormone

AV ;purified

AB CD36 is also known as GPIIIb, GPIV

SD 58

SD E54008M

LD USA BAL

EI DA>9002 CV>9007

CI ;catalog

SN Synonym>ACTH

- 66 -

1019119 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36 1.MW

1019119 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36 1.MW>88 kD

AN 1019119

SO J Cell Biol 1994;269:6011

SO J Cell Biol 1993;268:16179

DI P>Transduction Laboratories

DI 133 Venture Ct., Suite 5

DI Lexington, Ky 40511-9923

DI 1-606-259-1550

DI 1-800-227-4063

FE P>73 ;distributor

FE P>C23620 ;distributor

IM G>Homo sapiens CN>human SN>CD36 FS>amino acids 70-242

IM a.CC>protein

DO G>Mus musculus CN>mouse

PD ;IgG2a

RE 1.G>Homo sapiens 1.CN>human 1.SN>CD36 1.MW>88 kD

RE 1.a.CC>protein

RE 2.G>Rattus norvegicus 2.CN>Norway rat 2.SN>CD36

RE 2.MW>88 kD 2.a.CC>protein

RE 3.G>Gallus gallus 3.CN>chicken 3.SN>CD36 3.MW>88 kD

RE 3.a.CC>protein

AP ;Western blot ;immunofluorescence

AV ;purified

SD 73

SD C23620

LD USA JMJ

EI DA>9901

CI ;catalog

SN Synonym>CD36 1019119 SN Synonym>CD36

APPENDIX 2

DI is distributor
SD is standard designation

- 68 -

1022961 RE 1.SN>CD51 1.a.CC>differentiation

1022961 RE 1.SN>CD51 1.a.CC>differentiation

AN 1022961
DI P>Caltag Laboratories
DI 1849 Bayshore Blvd. #200
DI Burlingame, CA 94010
DI 1-650-652-0468
DI 1-800-874-4007
DE C>NGX-IV/110 ;distributor
DE P>MON1027 ;distributor
RE 1.SN>CD51 1.a.CC>differentiation

SD NGXIV110
LD USA MCM
EI DA>9805
CI ;catalog

- 69 -

1022017 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51/61 c

1022017 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51/61 c

AN 1022017

DI P>O.E.M. Concepts, Inc

DI 1889 Route 9, Bldg. 25, Unit 96

DI Toms River, NJ 08755 USA

DI 1-732-341-3570

DE C>289-12336 ;distributor

DE P>M2-L69 ;distributor

DO G>Mus musculus CN>mouse

RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51/61 complex

RE 1.a.CC>differentiation

AP ;cell surface marker

AV ;purified

AB Reactant#1: CD51/61 complex is also known as integrin alpha V
beta 3.

SD 28912336

SD M2L69

LD USA EJK

EI DA>9712

CI ;catalog

SN Synonym>CD51/61 complex 1022018 *****HB/HYBRID

- 70 -

1013413 RE 1.CE>platelet 1.SN>CD51 1.a.CC>differentiation

1013413 RE 1.CE>platelet 1.SN>CD51 1.a.CC>differentiation

AN 1013413
DI P>Medica
DI 2382 Camino Vida Roble, Suite I
DI Carlsbad, CA 92009 USA
DI 1-619-438-1886
DE C>706 ;developer
DE P>MON1130 ;distributor
PD ;IgG1
RE 1.CE>platelet 1.SN>CD51 1.a.CC>differentiation

SD MON1130
LD USA BAL
EI DA>9303
CI ;catalog

- 71 -

1024461 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51

1024461 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51

AN 1024461
DI P>Biogenesis Ltd.
DI 7 New Fields
DI Stinsford Road
DI Poole BH17 7NF, England
DI UK .
DI 44-1202 660006
DE C>13C2 ;distributor
DE P>2125-5108 ;distributor
DE P>2125-5114 ;distributor
DE P>2125-5119 ;distributor
DO G>Mus musculus CN>mouse
PD ;Ig
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51
RE 1.a.CC>differentiation
AP ;immunofluorescence
AV ;R-phycoerythrin conjugate ;fluorescein conjugate
AB CD51 is also known as integrin alpha V subunit and vitronectin
receptor
AB alpha subunit.
SD 13C2
SD 21255108
SD 21255114
SD 21255119
LD USA MCM
EI DA>9811
CI ;catalog

- 72 -

1017037 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51

1017037 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51

DI P>Zymed Laboratories Inc.
DI 458 Carlton Court
DI South San Francisco, CA 94080 USA
DI 1-800-874-4494 (toll free USA)
DI 1-415-871-4494
DE P>07-5103 ;distributor
DE P>NK1-M9 ;distributor
DO G>Mus musculus CN>mouse S>BALB/c
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51
RE 1.a.CC>differentiation 1.b.CC>protein
AP ;flow cytometry ;immunofluorescence
AV ;purified
SD 075103
SD NK1M9
LD USA JMJ
EI DA>9708
CI ;catalog

- 73 -

1009962 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51

1009962 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51

AN 1009962
DI P>Biodesign International
DI 105 York Street
DI Kennebunkport, ME 04043 USA
DI 1-207-985-1944
DI 2.P>Harlan Bioproducts for Science, Inc.
DI P.O. Box 29176
DI Indianapolis, IN 46229-0176
DI 1-317-894-7536
DI 1-800-9-SCIENCE
DE C>AMF7 ;developer
DE P>AMF7 ;distributor
DE P>P42770M ;distributor
DE 2.P>MCA 683 ;distributor
DO G>Mus musculus CN>mouse
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51
RE 1.a.CC>differentiation
AV ;purified ;2.purified
SD AMF7
SD MCA683
SD P42770M
LD USA BAL
EI DA>9103 CV>9104
CI ;catalog

- 74 -

1021411 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet

1021411 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD51

AN 1021411

DI P>Immunotech S.A.

DI Departement commercial

DI Luminy Case 915

DI 13288 Marseille Cedex 9, France

DI 33-91-41-41-38

DI 430246 F IMMTECH

DE C>69-6-5 ;distributor

DE P>1603 ;distributor

DO G>Mus musculus CN>mouse S>BALB/c O>spleen

PD ;IgG2a

RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD51

RE 1.a.CC>protein

AV ;purified

SD 1603

SD 6965

LD USA JMJ

EI DA>9707

CI ;catalog

- 75 -

1015384 RE 1.G>Mus musculus 1.CN>mous 1.SN>CD51

1015384 RE 1.G>Mus musculus 1.CN>mouse 1.SN>CD51

AN 1015384
DI P>PharMingen
DI 10975 Torreyana Road
DI San Diego, CA 92121 USA
DI 1-619-677-7737
DI 1-800-848-6227 (toll free USA)
DE C>H9.2B8 ;developer
DE P>01520D ;distributor
DE P>01521D ;distributor
DE P>01522D ;distributor
DE P>01525B ;distributor
DO G>Cricetulus sp. CN>hamster
IP G>Mus musculus CN>mouse
PD ;IgG
RE 1.G>Mus musculus 1.CN>mouse 1.SN>CD51
RE 1.a.CC>differentiation
AP ;flow cytometry ;immunofluorescence
AV ;biotin conjugate ;fluorescein conjugate
AV ;phycoerythrin conjugate ;purified
SD 01520D
SD 01521D
SD 01522D
SD 01524D
SD 01525B
SD H92B8
LD USA BAL
EI DA>9408
CI ;catalog

- 76 -

1023962 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51

1023962 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51

AN 1023962
SO Cell 1992;69:11-25
DI P>Ansell Corporation
DI 243 Third Street North
DI P.O. Box 87
DI Bayport, MN 55003 USA
DI 1-800-374-9523 (toll free USA)
DI 1-612-439-0835
DE C>P2W7 ;distributor
DE P>202-020 ;distributor
DE P>202-030 ;distributor
DE P>202-040 ;distributor
DE P>202-050 ;distributor
IM G>Homo sapiens CN>human O>eye PA>melanoma CD>V+B2 a.CC>neoplasm
DO G>Mus musculus CN>mouse
PD ;IgG1 ;kappa
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51
RE 1.a.CC>differentiation
AP ;immunoprecipitation ;flow cytometry ;frozen section
AV ;R-phycoerythrin conjugate ;biotin conjugate
AV ;fluorescein conjugate ;purified
SD 202020
SD 202030
SD 202040
SD 202050
SD P2W7
LD USA MCM
EI DA>9808
CI ;catalog

- 77 -

1023559 RE 1.G>Mus musculus 1.CN>mouse 1.SN>integrin

1023559 RE 1.G>Mus musculus 1.CN>mouse 1.SN>integrin alpha V

SO Biochemistry 1990;29:10191
SO Exp Cell Res 1993;205:25
DI P>Upstate Biotechnology, Inc.
DI 199 Saranac Avenue
DI Lake Placid, NY 12946 USA
DI 1-617-890-8845
DI 1-800-233-3991 (toll free USA) (sales)
DE P>05-437 ;distributor
DO G>Mus musculus CN>mouse
RE 1.G>Mus musculus 1.CN>mouse 1.SN>integrin alpha V
RE 1.MW>160 kD 1.a.CC>differentiation 1.b.CC>receptor
AP ;Western blot ;immunoprecipitation ;immunohistochemistry
AV ;ascites
AB Reactant is also known as vitronectin receptor alpha subunit and
CD51.
SD 05437
LD USA MCM
EI DA>9807
CI ;catalog

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1023927 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin

1023927 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V

AN 1023927
SO J Biol Chem 1994;269:6940
DI P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500(toll free USA)
DE C>P3G8 ;distributor
DE P>MAB1953 ;distributor
IM G>Homo sapiens CN>human O>lung PA>carcinoma a.CC>neoplasm
DO G>Mus musculus CN>mouse
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
RE 1.a.CC>differentiation 1.b.CC>receptor
AP ;immunocytology ;immunohistochemistry ;immunoprecipitation
AP ;flow cytometry ;ELISA ;FACS
AV ;purified
AB Reactant is also known as CD51 and vitronectin receptor alpha subunit.
AB Product reacts with all alpha V-containing integrin receptors.
AB Product will react with some lymphoid cell lines (B cells), many carcinoma and
AB melanoma cell lines and osteosarcomas.
SD MAB1953
SD P3G8
LD USA MCM
EI DA>9808
CI ;catalog

1015432 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51 1.FS

1015432 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51 1.FS>alpha subunit

AN 1015432
DI P>Biodesign International
DI 105 York Street
DI Kennebunkport, ME 04043 USA
DI 1-207-985-1944
DI 2.P>Caltag Laboratories
DI 1849 Bayshore Blvd. #200
DI Burlingame, CA 94010
DI 1-650-652-0468
DI 1-800-874-4007
DI 3.P>Cymbus Bioscience Limited
DI 2 Venture Road
DI Chilworth Research Center
DI Southampton, Hampshire SO1 7NS UK
DI 44-703-767178
DI 4.P>Endogen Inc.
DI 30 Commerce Way
DI Woburn, MA 01801-1059 USA
DI 1-781-937-0890
DI 5.P>Genosys Biotechnologies, Inc.
DI 1442 Lake Front Circle, Suite 185
DI The Woodlands, TX 77380-3600 USA
DI 1-713-363-3693
DI 1-800-234-5362 (toll free USA)
DI 6.P>Harlan Bioproducts for Science, Inc.
DI P.O. Box 29176
DI Indianapolis, IN 46229-0176
DI 1-317-894-7536
DI 1-800-9-SCIENCE
DI 7.P>Lampire Biological Laboratories
DI P.O. Box 270
DI Pipersville, PA 18947 USA
DI 1-215-795-2838
DI 8.P>PharMingen
DI 10975 Torreyana Road
DI San Diego, CA 92121 USA
DI 1-619-677-7737
DI 1-800-848-6227 (toll free USA)
DI 9.P>T Cell Diagnostics, Inc.
DI 6 Gill Street
DI Woburn, MA 01801-1721 USA
DI 1-800-624-4021
DI 1-617-937-9587
DE C>23C6 ;developer
DE P>23C6 ;distributor
DE P>P54490M ;distributor
DE 2.P>23C6 ;distributor
DE 2.P>MON1167 ;distributor
DE 3.P>23C6 ;distributor
DE 3.P>CBL490 ;distributor
DE 4.P>23C6 ;distributor
DE 4.P>MA-5100 ;distributor
DE 5.P>23C6 ;distributor
DE 5.P>AM-19-760 ;distributor
DE 6.P>23C6 ;distributor
DE 6.P>MCA-757 ;discontinued designation

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DE 6.P>MCA757G ;distributor
DE 7.P>23C6 ;distributor
DE 7.P>LBL 590 ;distributor
DE 8.P>23C6 ;distributor
DE 8.P>31561A ;distributor
DE 8.P>31564X ;distributor
DE 9.P>23C6 ;distributor
DE 9.P>IA1S04 ;distributor
DO G>Mus musculus CN>mouse
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD51 1.FS>alpha subunit
RE 1.MW>125 kD 1.a.CC>differentiation
AV ;purified ;4.purified ;6.purified ;8.fluorescein conjugate
AV ;8.purified ;9.supernatant
SD 23C6
SD 31561A
SD 31564X
SD AM19760
SD CBL490
SD IA1S04
SD LBL590
SD MA5100
SD MCA757
SD MCA757G
SD MON1167
SD P54490M
LD USA BAL
EI DA>9706
CI ;catalog

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1011348 RE 1.G>Homo sapiens 1.CN>human 1.SN>vitronect

1011348 RE 1.G>Homo sapiens 1.CN>human 1.SN>vitronectin receptor

AN 1011348

DI P>Chemicon International, Inc.

DI 28835 Single Oak Dr.

DI Temecula, CA 92590 USA

DI 1-909-676-8080

DI 1-800-437-7500(toll free USA)

DE P>CLB-706 ;distributor

DE P>MAB1980 ;distributor

RE 1.G>Homo sapiens 1.CN>human 1.SN>vitronectin receptor

RE 1.a.CC>receptor

AV ;purified

AB beta subunit of vitronectin receptor referred to as CD51 also

AB Reactant#1: vitronectin receptor beta subunit syn. for CD51

SD CLB706

SD MAB1980

LD USA BAL

EI DA>9107 CV>9108

CI ;catalog

SN Synonym>vitronectin receptor

APPENDIX 3

DI is distributor
SD is standard designation

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Beta 5

1019741 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 5
AN 1019741
DI P>Upstate Biotechnology, Inc.
DI 199 Saranac Avenue
DI Lake Placid, NY 12946 USA
DI 1-617-890-8845
DI 1-800-233-3991 (toll free USA) (sales)
DE C>B5-IVF2 ;distributor
DE P>05-283 ;distributor
DO G>Mus musculus CN>mouse S>BALB/c O>spleen
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 5
RE 1.a.CC>protein
AP ;immunocytochemistry ;blocks cell adhesion
AV ;ascites
SD 05283
SD B5IVF2
LD USA JMJ
EI DA>9611
CI ;catalog

- 84 -

Beta 3

1011332 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
AN 1011332
DI P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500(toll free USA)
DE P>MAB1974 ;distributor
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
RE 1.a.CC>receptor
AV ;ascites
SD MAB1974
LD USA BAL
EI DA>9107 CV>9108
CI ;catalog

- 85 -

1014236 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
AN 1014236
AU Ylanne J
SO Blood 1989;72:1478-86
SO Blood 1990;76:570-7
DI P>Biohit Oy
DI Verkkosaarencatu 4
DI 00580 Helsinki, Finland
DI 358-0-773-2900
DI 2.P>Harlan Bioproducts for Science, Inc.
DI P.O. Box 29176
DI Indianapolis, IN 46229-0176
DI 1-317-894-7536
DI 1-800-9-SCIENCE
DI 3.P>ICN Biomedicals
DI Biomedical Research Products
DI 3300 Hyland Avenue
DI Costa Mesa, CA 92626
DI 1-800-854-0530 (toll free USA)
DI 1-714-545-0100
DI 4.P>Locus Genex Oy
DI Verkkosaarencatu 4
DI 00580 Helsinki, Finland
DI 358-9-773-861
DE C>BB10 ;developer
DE P>M-9006000 ;distributor
DE P>M-9006100 ;distributor
DE 2.P>MCA-781 ;distributor
DE 2.P>bb10 ;distributor
DE 3.P>69-323-1 ;distributor
DE 3.P>69-323-2 ;distributor
DE 3.P>BB10 ;distributor
DE 4.P>BB10 ;distributor
DE 4.P>M-9006000 ;distributor
DE 4.P>M-9006100 ;distributor
IM G>Homo sapiens CN>human SN>CD41 ;purified a.CC>differentiation
RM ;in vivo
DO G>Mus musculus CN>mouse S>BALB/c O>spleen
IP G>Mus musculus CN>mouse PA>myeloma
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
RE 1.a.CC>receptor
RE 2.G>Homo sapiens 2.CN>human 2.SN>platelet GPIIIa
RE 2.a.CC>blood coagulation factor
AP ;immunoassay ;not paraffin section ;immunohistochemical staining
AP ;immunoblotting
AV ;purified ;2.purified ;3.purified ;4.purified
SD 693231
SD 693232
SD BB10
SD M9006000
SD M9006100
SD MCA781
LD USA BAL

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EI DA>9711

SN Synonym>CD41 1014236 SN Synonym>CD41

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1014281 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
AN 1014281
DI P>Bio-Science Products AG
DI Gerliswilstrasse 43
DI Postfach 1173
DI CH-6020 Emmenbrucke, Switzerland
DI 41-555875
DE P>0121022 ;distributor
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
RE 1.a.CC>receptor
RE 2.G>Homo sapiens 2.CN>human 2.SN>CD41
RE 2.a.CC>differentiation
AP ;immunoassay ;immunoblotting ;not paraffin section
AP ;immunohistochemical staining
LD USA BAL
EI DA>9305
CI ;catalog
SN Synonym>CD41 1014281 SN Synonym>CD41

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1019109 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
AN 1019109
SO J Cell Biol 1993;122:223
SO J Cell Biol 1993;121:689
DI P>Transduction Laboratories
DI 133 Venture Ct., Suite 5
DI Lexington, Ky 40511-9923
DI 1-606-259-1550
DI 1-800-227-4063
DE P>26 ;distributor
DE P>I19620 ;distributor
IM G>Mus musculus CN>mouse SN>integrin beta 3 FS>amino acids 16-223
IM a.CC>protein
DO G>Mus musculus CN>mouse
PD ;IgM
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
RE 1.MW>90 kD 1.a.CC>protein
RE 2.G>Canis familiaris 2.CN>dog 2.SN>integrin beta 3
RE 2.MW>90 kD 2.a.CC>protein
RE 3.G>Rattus norvegicus 3.CN>Norway rat 3.SN>integrin beta 3
RE 3.MW>90 kD 3.a.CC>protein
RE 4.G>Mus musculus 4.CN>mouse 4.SN>integrin beta 3
RE 4.MW>90 kD 4.a.CC>protein
RE 5.G>Gallus gallus 5.CN>chicken 5.SN>integrin beta 3
RE 5.MW>90 kD 5.a.CC>protein
AP ;Western blot
AV ;purified
SD 26
SD I19620
LD USA JMJ
EI DA>9705
CI ;catalog

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1023930 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
AN 1023930
SO Cell 1986;45:269-80
DI P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500(toll free USA)
DE C>25E11 ;distributor
DE P>MAB1957 ;distributor
IM T>blood CE>mononuclear cell CS>activated
DO G>Mus musculus CN>mouse
PD ;IgG2a
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin beta 3
RE 1.a.CC>differentiation 1.b.CC>receptor
RE 2.SN>CD41/CD61 complex 2.a.CC>protein
RE 3.G>Homo sapiens 3.CN>human 3.CE>monoblast 3.PA>leukemia
RE 3.CD>U937 3.SN>CD41/CD61 complex 3.a.CC>protein
AP ;Western blot ;immunocytology ;immunoprecipitation
AP ;flow cytometry
AV ;purified a.PM>protein A chromatography
AB Integrin beta 3 is also known as CD61, GPIIIa, and vitronectin
AB receptor beta chain.
SD 25E11
SD MAB1957
LD USA MCM
EI DA>9808
CI ;catalog

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1024059 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
AN 1024059
DI P>PharMingen
DI 10975 Torreyana Road
DI San Diego, CA 92121 USA
DI 1-619-677-7737
DI 1-800-848-6227 (toll free USA)
DE C>VI-PL2 ;distributor
DE P>33821 ;distributor
DE P>33824 ;distributor
DE P>33825 ;distributor
DO G>Mus musculus CN>mouse
PD ;IgG1 ;kappa
RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
RE 1.MW>105 kD 1.a.CC>differentiation 1.b.CC>glycoprotein
RE 2.G>Homo sapiens 2.CN>human 2.CE>megakaryocyte 2.SN>CD61
RE 2.MW>105 kD 2.a.CC>differentiation 2.b.CC>glycoprotein
RE 3.G>Homo sapiens 3.CN>human 3.CE>osteoclast 3.SN>CD61
RE 3.MW>105 kD 3.a.CC>differentiation 3.b.CC>glycoprotein
RE 4.G>Homo sapiens 4.CN>human 4.T>endothelium 4.SN>CD61
RE 4.MW>105 kD 4.a.CC>differentiation 4.b.CC>glycoprotein
XR 1.G>Canis sp. 1.CN>dog 1.SN>CD61 1.a.CC>differentiation
XR 1.b.CC>glycoprotein
XR 2.G>Felis sp. 2.CN>cat 2.SN>CD61 2.a.CC>differentiation
XR 2.b.CC>glycoprotein
NR 1.G>Sus sp. 1.CN>swine 1.SN>CD61 1.a.CC>differentiation
NR 1.b.CC>glycoprotein
AP ;acetone fixed ;frozen section
AV ;R-phycoerythrin conjugate ;biotin conjugate
AV ;fluorescein conjugate ;purified
AB CD61 is also known as integrin beta 3 subunit.
SD 33821
SD 33824
SD 33825
SD VIPL2
LD USA MCM
EI DA>9809
CI ;catalog

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1011347 RE 1.G>Homo sapiens 1.CN>human 1.SN>vitronectin receptor
AN 1011347
DI P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500 (toll free USA)
DE P>MAB1984 ;distributor
RE 1.G>Homo sapiens 1.CN>human 1.SN>vitronectin receptor
RE 1.a.CC>receptor
AB Reactant#1 vitronectin receptor alpha subunit syn. for CD61
SD MAB1984
LD USA BAL
EI DA>9107 CV>9108
CI ;catalog
SN Synonym>vitronectin receptor

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1012445 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD61
AN 1012445
DI P>Cymbus Bioscience Limited
DI 2 Venture Road
DI Chilworth Research Center
DI Southampton, Hampshire SO1 7NS UK
DI 44-703-767178
DE C>Thromb/1 ;developer
DE P>CBL 458 ;discontinued designation
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD61
RE 1.a.CC>differentiation
SD THROMB1
LD USA BAL
EI DA>9110 CV>9111
CI ;catalog

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1013416 RE 1.CE>platelet 1.SN>CD61 1.a.CC>differentiation
AN 1013416
DI P>Caltag Laboratories
DI 1849 Bayshore Blvd. #200
DI Burlingame, CA 94010
DI 1-650-652-0468
DI 1-800-874-4007
DI 2.P>Medica
DI 2382 Camino Vida Roble, Suite I
DI Carlsbad, CA 92009 USA
DI 1-619-438-1886
DI 3.P>Sigma Chemical Company
DI P.O. Box 14508
DI St. Louis, MO 63178 9916 USA
DI 1-800-325-3010 (toll free USA)
DI 1-314-771-5750
DE C>BL-E6 ;developer
DE P>BL-E6 ;distributor
DE P>MHCD6101 ;distributor
DE P>MHCD6101-4 ;distributor
DE P>MHCD6115 ;distributor
DE P>MHCD6115-4 ;distributor
DE P>MON1051 ;distributor
DE 2.P>MON1051 ;distributor
DE 3.P>BL-E6 ;distributor
DE 3.P>C4321 ;distributor
DE 3.P>F7902 ;distributor
DO G>Mus musculus CN>mouse
PD ;IgG1
RE 1.CE>platelet 1.SN>CD61 1.a.CC>differentiation
RE 2.CE>megakaryocyte 2.SN>CD61 2.a.CC>differentiation
AP ;frozen section
AV ;biotin conjugate ;fluorescein conjugate
AV ;3.fluorescein conjugate
SD BLE6
SD C4321
SD F7902
SD MHCD6101
SD MHCD61014
SD MHCD6115
SD MHCD61154
SD MON1051
LD USA BAL
EI DA>9803
CI ;catalog

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1013417 RE 1.CE>platelet 1.SN>CD61 1.a.CC>differentiation
AN 1013417
DI P>Caltag Laboratories
DI 1849 Bayshore Blvd. #200
DI Burlingame, CA 94010
DI 1-650-652-0468
DI 1-800-874-4007
DI 2.P>Medica
DI 2382 Camino Vida Roble, Suite I
DI Carlsbad, CA 92009 USA
DI 1-619-438-1886
DE C>CRC54 ;developer
DE P>CRC54 ;distributor
DE P>MON1147 ;distributor
DE 2.P>MON1147 ;distributor
PD ;IgG1
RE 1.CE>platelet 1.SN>CD61 1.a.CC>differentiation
RE 2.CE>megakaryocyte 2.SN>CD61 2.a.CC>differentiation
AP ;frozen section
SD MON1147
LD USA BAL
EI DA>9303
CI ;catalog

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1014017 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD61
DI P>Diagast Laboratories
DI 59, rue de Trevis-B.P. 2034
DI 59014 Lille Cedex, France
DI 33-20-52-68-00
DI DIAGAST (042) 160716F
DE P>16101V ;distributor
DE P>16103A ;distributor
DE P>16105E ;distributor
PD ;IgG
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD61
RE 1.a.CC>differentiation
AV ;fluorescein conjugate ;phycoerythrin conjugate ;purified
SD 16101V
SD 16103A
SD 16105E
LD USA BAL
EI DA>9304
CI ;catalog

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1014618 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
AN 1014618
DI P>BioSource International
DI 820 Flynn Roa
DI Camarillo, CA 93012 USA
DI 1-800-242-0607(toll free USA)
DI 1-805-987-0086
DI 2.P>Cymbus Bioscience Limited
DI 2 Venture Road
DI Chilworth Research Center
DI Southampton, Hampshire SO1 7NS UK
DI 44-703-767178
DI 3.P>Endogen Inc.
DI 30 Commerce Way
DI Woburn, MA 01801-1059 USA
DI 1-781-937-0890
DI 4.P>Genosys Biotechnologies, Inc.
DI 1442 Lake Front Circle, Suite 185
DI The Woodlands, TX 77380-3600 USA
DI 1-713-363-3693
DI 1-800-234-5362 (toll free USA)
DI 5.P>Harlan Bioproducts for Science, Inc.
DI P.O. Box 29176
DI Indianapolis, IN 46229-0176
DI 1-317-894-7536
DI 1-800-9-SCIENCE
DI 6.P>Lampire Biological Laboratories
DI P.O. Box 270
DI Pipersville, PA 18947 USA
DI 1-215-795-2838
DI 7.P>Novocastra Laboratories Ltd.
DI 24 Claremont Place
DI Newcastle upon Tyne NE2 4AA, UK
DI 44-0191 222 8550
DI 8.P>Southern Biotechnology Associates, Inc.
DI P.O. Box 26221
DI Birmingham, AL 35260 USA
DI 1-800-722-2255 (toll free USA)
DI 1-205-945-1774
DI 9.P>T Cell Diagnostics, Inc.
DI 6 Gill Street
DI Woburn, MA 01801-1721 USA
DI 1-800-624-4021
DI 1-617-937-9587
DE C>PM 6/13 ;developer
DE P>AHS6101 ;distributor

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DE P>AHS6107 ;distributor
DE P>AHS6108 ;distributor
DE P>CS-CD61-CF ;discontinued designation
DE P>CS-CD61-FI ;discontinued designation
DE P>CS-CD61-PE ;discontinued designation
DE 2.P>CBL479 ;distributor
DE 2.P>PM6/13 ;distributor
DE 3.P>MA-6100 ;distributor
DE 3.P>PM6/13 ;distributor
DE 4.P>AM-19-705 ;distributor
DE 4.P>PM6/13 ;distributor
DE 5.P>MCA-728 ;distributor
DE 5.P>MCA-728F ;distributor
DE 5.P>MCA-728PE ;distributor
DE 5.P>PM6/13 ;distributor
DE 6.P>LBL 579 ;distributor
DE 6.P>PM6/13 ;distributor
DE 7.P>NCL-CD61 ;distributor
DE 7.P>PM6/13 ;distributor
DE 8.P>9470-01 ;distributor
DE 8.P>9470-02 ;distributor
DE 8.P>9470-08 ;distributor
DE 8.P>PM6/13 ;distributor
DE 9.P>IA1S09 ;distributor
DE 9.P>PM6/13 ;distributor
DO G>Mus musculus CN>mouse
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
RE 1.MW>90 kD 1.a.CC>differentiation
RE 2.G>Homo sapiens 2.CN>human 2.T>plasma 2.SN>CD61
RE 2.MW>90 kD 2.a.CC>differentiation
RE 3.G>Homo sapiens 3.CN>human 3.T>plasma
RE 3.PA>unspecified neoplasm 3.SN>CD61 3.MW>90 kD
RE 3.a.CC>neoplasm 3.b.CC>differentiation
AV ;fluorescein conjugate ;phycoerythrin conjugate ;purified
AV ;3.purified ;5.fluorescein conjugate
AV ;5.phycoerythrin conjugate ;5.purified ;8.biotin conjugate
AV ;8.fluorescein conjugate ;8.purified ;9.purified
SD 947001
SD 947002
SD 947008
SD AHS6101
SD AHS6107
SD AHS6108
SD AM19705
SD CBL479
SD CSCD61CF

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SD CSCD61FI
SD CSCD61PE
SD IA1S09
SD LBL579
SD MA6100
SD MCA728
SD MCA728F
SD MCA728PE
SD NCLCD61
SD PM613
LD USA BAL
EI DA>9904
CI ;catalog

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1017042 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
AN 1017042
DI P>Becton Dickinson Immunocytometry Systems
DI 2350 Qume Drive
DI San Jose, CA 95131-1807
DI 1-800-223-8226 (toll free USA)
DI 1-408-954-2347
DE P>348090 ;distributor
DE P>348093 ;distributor
DE P>559936 ;distributor
DE P>RUU-PL7F12 ;distributor
DO G>Mus musculus CN>mouse S>BALB/c
PD ;IgG1 ;kappa
RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
RE 1.a.CC>differentiation 1.b.CC>protein
AP ;immunofluorescence ;immunoprecipitation
AV ;fluorescein conjugate ;purified
SD 348090
SD 348093
SD 559936
SD RUUPL7F12
LD USA JMJ
EI DA>9505
CI ;catalog

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1017635 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD61
AN 1017635
DI P>Novocastra Laboratories Ltd.
DI 24 Claremont Place
DI Newcastle upon Tyne NE2 4AA, UK
DI 44-0191 222 8550
DE P>NCL-CD61 ;distributor
DE P>PM6/13 ;distributor
DO G>Mus musculus CN>mouse
AS ;immunohistochemical staining
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD61
RE 1.a.CC>differentiation
AP ;frozen section
AV ;ascites
SD NCLCD61
SD PM613
LD USA BAL
EI DA>9904
CI ;catalog

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1019745 RE 1.G>Homo sapiens 1.CN>human 1.SN>CD61
AN 1019745
DI P>Upstate Biotechnology, Inc.
DI 199 Saranac Avenue
DI Lake Placid, NY 12946 USA
DI 1-617-890-8845
DI 1-800-233-3991 (toll free USA) (sales)
DE P>05-275 ;distributor
DO G>Mus musculus CN>mouse S>BALB/c O>spleen
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>CD61
RE 1.a.CC>differentiation 1.b.CC>protein
AP ;immunocytochemistry
AV ;ascites
SD 05275
LD USA JMJ
EI DA>9611
CI ;catalog

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1020416 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
AN 1020416
DI P>PanVera Corporation
DI 545 Science Drive
DI Madison, WI 53711 USA
DI 1-800-791-1400
DI 1-608-233-9450
DE C>PL8-5 ;distributor
DE P>TAK M068 ;distributor
DO G>Mus musculus CN>mouse S>BALB/c O>spleen
PD ;IgG
RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
RE 1.a.CC>differentiation 1.b.CC>protein
AV ;purified
SD PL85
SD TAKM068
LD USA JMJ
EI DA>9702
CI ;catalog

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1020417 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
AN 1020417
DI P>PanVera Corporation
DI 545 Science Drive
DI Madison, WI 53711 USA
DI 1-800-791-1400
DI 1-608-233-9450
DE C>PL11-7 ;distributor
DE P>TAK M069 ;distributor
DO G>Mus musculus CN>mouse S>BALB/c O>spleen
PD ;IgG
RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
RE 1.a.CC>differentiation 1.b.CC>protein
AV ;purified
SD PL117
SD TAKM069
LD USA JMJ
EI DA>9702
CI ;catalog

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1021005 RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
AN 1021005
DI P>Cortex Biochem, Inc.
DI 1933 Davis Street, Suite 321
DI San Leandro, CA 94577 USA
DI 1-800-888-7713 (toll free USA)
DI 1-510-568-3911 (technical)
DE P>CR1153 ;distributor
RE 1.G>Homo sapiens 1.CN>human 1.CE>platelet 1.SN>CD61
RE 1.a.CC>differentiation
RE 2.G>Homo sapiens 2.CN>human 2.CE>megakaryocyte 2.SN>CD61
RE 2.a.CC>differentiation
LD USA CLB
EI DA>9704
CI ;catalog

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1011328 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
AN 1011328
DI P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500(toll free USA)
DI 2.P>Life Technologies, Inc.
DI 8400 Helgerman Ct.
DI P.O. Box 6009
DI Gaithersburg, MD 20884-9980 USA
DI 1-301-840-8000
DI 1-800-828-6686 (Toll free USA)
DE P>MAB1958 ;distributor
DE P>VNR147 ;distributor
DE 2.P>12084-018 ;distributor
DE 2.P>VNR147 ;distributor
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
RE 1.a.CC>receptor
AV ;ascites ;2.ascites
SD 12084018
SD MAB1958
SD VNR147
LD USA BAL
EI DA>9706 CV>9108
CI ;catalog

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1011329 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
AN 1011329
DI P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500(toll free USA)
DI 2.P>Life Technologies, Inc.
DI 8400 Helgerman Ct.
DI P.O. Box 6009
DI Gaithersburg, MD 20884-9980 USA
DI 1-301-840-8000
DI 1-800-828-6686 (Toll free USA)
DE P>MAB1960 ;distributor
DE P>VNR139 ;distributor
DE 2.P>12085-015 ;distributor
DE 2.P>VNR139 ;distributor
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
RE 1.a.CC>receptor
AV ;ascites ;2.ascites
SD 12085015
SD MAB1960
SD VNR139
LD USA BAL
EI DA>9706 CV>9108
CI ;catalog

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1011842 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
AN 1011842
DI P>Biogenesis Ltd.
DI 7 New Fields
DI Stinsford Road
DI Poole BH17 7NF, England
DI UK
DI 44-1202 660006
DE C>1U4/1 ;developer
DE P>5355-2505 ;distributor
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
RE 1.a.CC>protein
AP ;immunoblotting .
AV ;ascites
SD 1U41
SD 53552505
LD USA BAL
EI DA>9109 CV>9110
CI ;catalog

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1011843 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
AN 1011843
DI P>Biogenesis Ltd.
DI 7 New Fields
DI Stinsford Road
DI Poole BH17 7NF, England
DI UK
DI 44-1202 660006
DE C>1U3/0 ;developer
DE P>5355-2515 ;distributor
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
RE 1.a.CC>protein
AP ;immunofluorescence
AV ;ascites
SD 1U30
SD 53552515
LD USA BAL
EI DA>9109 CV>9110
CI ;catalog

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1012705 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
AN 1012705
DI P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500 (toll free USA)
DE P>LM142 ;distributor
DE P>MAB1978 ;distributor
PD ;IgG
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
RE 1.a.CC>protein
AV ;ascites
SD LM142
SD MAB1978
LD USA BAL
EI DA>9112 CV>9201
CI ;catalog

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1013223 RE 1.SN>integrin alpha V 1.a.CC>protein
AN 1013223
DI P>American Qualex International, Inc.
DI 920-A Calle Negocio St.
DI San Clemente, CA 92673
DI 1-714-521-3753
DI 1-800-772-1776 (toll free USA)
DE P>M2580 ;distributor
RE 1.SN>integrin alpha V 1.a.CC>protein
SD M2580
LD USA BAL
EI DA>9204 CV>9204
CI ;catalog

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1013510 RE 1.G>Homo sapiens 1.CN>human 1.CE>endothelial cell
AN 1013510
AU Freed E
SO EMBO J 1989;8:2955
DI P>Calbiochem Novabiochem International
DI P.O. Box 12087
DI La Jolla, CA 92039-2087
DI 1-800-854-3417(toll free USA)
DI 1-619-450-9600
DE P>407281 ;distributor
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.CE>endothelial cell
RE 1.SN>integrin alpha V 1.FS>type 1 1.a.CC>protein
RE 2.G>Homo sapiens 2.CN>human 2.SN>vitronectin
RE 2.a.CC>protein
RE 3.G>Homo sapiens 3.CN>human 3.SN>fibrinogen
RE 3.a.CC>protein
RE 4.G>Homo sapiens 4.CN>human 4.SN>osteopontin
RE 4.a.CC>protein
RE 5.G>Homo sapiens 5.CN>human 5.SN>von Willebrand factor
RE 5.a.CC>protein
RE 6.G>Homo sapiens 6.CN>human 6.O>bone 6.SN>sialoprotein I
RE 6.a.CC>protein
AP ;ELISA ;immunofluorescence ;immunoprecipitation
AV ;ascites ;lyophilized
SD 407281
LD USA BAL
EI DA>9303
CI ;catalog
SN Synonym>fibrinogen
SN Synonym>von Willebrand factor

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1013511 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
AN 1013511
DI P>Calbiochem Novabiochem International
DI P.O. Box 12087
DI La Jolla, CA 92039-2087
DI 1-800-854-3417(toll free USA)
DI 1-619-450-9600
DE P>407282 ;distributor
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
RE 1.FS>type 2 1.a.CC>protein
RE 2.G>Homo sapiens 2.CN>human 2.SN>vitronectin
RE 2.a.CC>protein
RE 3.G>Homo sapiens 3.CN>human 3.SN>fibrinogen
RE 3.a.CC>protein
RE 4.G>Homo sapiens 4.CN>human 4.SN>osteopontin
RE 4.a.CC>protein
RE 5.G>Homo sapiens 5.CN>human 5.SN>von Willebrand factor
RE 5.a.CC>protein
RE 6.G>Homo sapiens 6.CN>human 6.O>bone 6.SN>sialoprotein I
RE 6.a.CC>protein
AP ;ELISA ;immunoblotting
AV ;ascites ;lyophilized
SD 407282
LD USA BAL
EI DA>9303
CI ;catalog
SN Synonym>fibrinogen
SN Synonym>von Willebrand factor

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1014225 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V beta 5
AN 1014225
AU Wayner EA
SO J Cell Biol 1991;113:919
DI P>Becton Dickinson Immunocytometry Systems
DI 2350 Qume Drive
DI San Jose, CA 95131-1807
DI 1-800-223-8226 (toll free USA)
DI 1-408-954-2347
DI 2.P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500(toll free USA)
DI 3.P>Life Technologies, Inc.
DI 8400 Helgerman Ct.
DI P.O. Box 6009
DI Gaithersburg, MD 20884-9980 USA
DI 1-301-840-8000
DI 1-800-828-6686 (Toll free USA)
DI 4.P>Telios Pharmaceuticals, Inc.
DI 4757 Nexus Centre Drive
DI San Diego, CA 92121 USA
DI 1-619-622-2650
DE C>P1F6 ;developer
DE P>550045 ;distributor
DE P>P1F6 ;distributor
DE 2.P>MAB1961 ;distributor
DE 2.P>P1F6 ;distributor
DE 3.P>12078-010 ;distributor
DE 3.P>P1F6 ;distributor
DE 4.P>A035 ;distributor
DO G>Mus musculus CN>mouse
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V beta 5
RE 1.a.CC>receptor
AP ;immunofluorescence ;immunoprecipitation
AV ;2.ascites ;3.ascites ;4.ascites
SD 12078010
SD 550045
SD A035
SD MAB1961
SD P1F6
LD USA BAL
EI DA>9706
CI ;catalog

- 114 -

1014277 RE 1.G>Homo sapiens 1.CN>human 1.CE>endothelial cell
AN 1014277
DI P>Bio-Science Products AG
DI Gerliswilstrasse 43
DI Postfach 1173
DI CH-6020 Emmenbrucke, Switzerland
DI 41-555875
DE P>0121005 ;distributor
RE 1.G>Homo sapiens 1.CN>human 1.CE>endothelial cell
RE 1.SN>integrin alpha V 1.a.CC>receptor
RE 2.G>Homo sapiens 2.CN>human 2.PA>carcinoma
RE 2.SN>integrin alpha V 2.a.CC>neoplasm 2.b.CC>receptor
NR 1.G>Homo sapiens 1.CN>human 1.SN>fibronectin receptor
NR 1.a.CC>receptor
NR 2.G>Homo sapiens 2.CN>human 2.SN>CD41
NR 2.a.CC>differentiation
AP ;ELISA ;immunofluorescence ;not immunoblotting
AP ;immunoprecipitation
LD USA BAL
EI DA>9305
CI ;catalog
SN Synonym>CD41 1014277 SN Synonym>CD41
SN Synonym>fibronectin receptor

- 115 -

1014278 RE 1.G>Homo sapiens 1.CN>human 1.CE>endothelial cell
AN 1014278
DI P>Bio-Science Products AG
DI Gerliswilstrasse 43
DI Postfach 1173
DI CH-6020 Emmenbrucke, Switzerland
DI 41-555875
DE P>0121006 ;distributor
AS ;immunoblot
RE 1.G>Homo sapiens 1.CN>human 1.CE>endothelial cell
RE 1.SN>integrin alpha V 1.a.CC>receptor
RE 2.G>Homo sapiens 2.CN>human 2.PA>carcinoma
RE 2.SN>integrin alpha V 2.a.CC>neoplasm 2.b.CC>receptor
AP ;immunoassay
LD USA BAL
EI DA>9305
CI ;catalog

- 116 -

1023559 RE 1.G>Mus musculus 1.CN>mouse 1.SN>integrin alpha V
SO Biochemistry 1990;29:10191
SO Exp Cell Res 1993;205:25
DI P>Upstate Biotechnology, Inc.
DI 199 Saranac Avenue
DI Lake Placid, NY 12946 USA
DI 1-617-890-8845
DI 1-800-233-3991 (toll free USA) (sales)
DE P>05-437 ;distributor
DO G>Mus musculus CN>mouse
RE 1.G>Mus musculus 1.CN>mouse 1.SN>integrin alpha V
RE 1.MW>160 kD 1.a.CC>differentiation 1.b.CC>receptor
AP ;Western blot ;immunoprecipitation ;immunohistochemistry
AV ;ascites
AB Reactant is also known as vitronectin receptor alpha subunit and
CD51.
SD 05437
LD USA MCM
EI DA>9807
CI ;catalog

- 117 -

1023927 RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
AN 1023927
SO J Biol Chem 1994;269:6940
DI P>Chemicon International, Inc.
DI 28835 Single Oak Dr.
DI Temecula, CA 92590 USA
DI 1-909-676-8080
DI 1-800-437-7500 (toll free USA)
DE C>P3G8 ;distributor
IM G>Homo sapiens CN>human O>lung PA>carcinoma a.CC>neoplasm
DO G.Mus musculus CN>mouse
PD ;IgG1
RE 1.G>Homo sapiens 1.CN>human 1.SN>integrin alpha V
RE 1.a.CC>differentiation 1.b.CC>receptor
AP ;immunocytology ;immunohistochemistry ;immunoprecipitation
AP ;flow cytometry ;ELISA ;FACS
AV ;purified
AB Reactant is also known as CD51 and vitronectin receptor alpha subunit.
AB Product reacts with all alpha V-containing integrin receptors.
AB Product will react with some lymphoid cell lines (B cells), many
AB carcinoma and melanoma cell lines and osteosarcomas.
SD MAB1953
SD P3G8
LD USA MCM
EI DA>9808
CI ;catalog

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CLAIMS:

1. A method of treating mammalian dendritic cells in vitro to induce immune tolerance therein
5 which comprises exposing said cells to an agonist of the cell surface receptors CD36 and/or CD51 as expressed on mammalian dendritic cells.
2. A method as claimed in claim 1 wherein said
10 dendritic cells are human or mouse.
3. A method as claimed in claim 1 or 2 wherein said agonist is a molecule identified as such by any one of the methods of claims 15 to 32.
15
4. A method as claimed in claim 1 or 2 wherein said agonist is selected from: an antibody with an affinity for an epitope of CD36, an antibody with an affinity for an epitope of CD51, the Plasmodium
20 falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1, thrombospondin, apoptotic cells or a negatively charged phospholipid.
5. A method as claimed in claim 1 or 2 which
25 comprises exposing said dendritic cells to two or more of the agonists of claim 4.
6. A method as claimed in claim 4 or claim 5 wherein the CD36 agonist any one of the antibodies
30 listed in Appendix 1.
7. A method as claimed in claim 4 or claim 5 wherein the CD51 agonist is any one of the antibodies
35 listed in Appendix 2.
8. A method as claimed in claim 4 or claim 5

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wherein the pf-EMP-1 active binding domain comprises the amino acid sequence shown in Figure 2.

5 9. A method as claimed in any one of claims 40 to 47 wherein said cells are exposed to an antigenic material.

10 10. A method as claimed in claim 9 wherein said antigenic material is an auto-antigen associated with a particular auto-immune disease.

15 11. A method as claimed in claim 9 or 10 wherein the cells so produced are subsequently matured by exposure to an immune stimulus.

20 12. A method as claimed in any of claims 1 to 11 wherein said dendritic cells are prepared from human peripheral blood and or derived from CD34+ stem cells or monocytes.

25 13. A dendritic cell preparation obtainable by the method of any of claims 1 to 12 for use as a medicament.

30 14. A dendritic cell preparation obtainable by the method of any of claims 1 to 12 for use in inducing peripheral immune tolerance in a human.

35 15. A method of identifying a molecule which is an agonist of cell surface receptor CD36 and/or CD51 as expressed by mammalian dendritic cells which method comprises:

 a) exposing immature mammalian dendritic cells to the molecule to be tested,

 b) exposing said immature dendritic cells to an immune stimulus and

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c) determining the degree of maturation
manifested by said dendritic cells,

wherein impaired maturation in response to the
immune stimulus is an indication that said molecule
5 under test is a CD36 and/or CD51 agonist.

16. A method as claimed in claim 15 wherein
said dendritic cells are human cells or mouse cells.

10 17. A method as claimed in claim 15 or 16
wherein maturation of said dendritic cells is
determined by examining the antigen-presenting ability
of said cells.

15 18. A method as claimed in any of claims 15 to
17 wherein maturation of said dendritic cells is
determined by examining said cells for expression of
at least one cell surface antigen whose level of
expression is enhanced in response to an immune
20 stimulus.

19. A method as claimed in claim 18 wherein
maturation of said dendritic cells is determined by
measuring the level of expression of one or more of
25 the following panel of antigens:
HLA DR, CD54, CD40, CD83 and CD86.

20. A method as claimed in claim 19 wherein
said cells are also examined for expression of CD80.
30

21. A method as claimed in any one of claims 18
to 20 wherein the level of expression of said antigens
is detected using a labelled antibody.

35 22. A method as claimed in 15 or 16 wherein
maturation of said dendritic cells is determined by
measuring said cells' ability to induce T-cell

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proliferation.

23. The method of claim 15 or 16 wherein
maturation of said dendritic cells is determined by
5 quantifying the level of cytokines secreted from said
cells.

24. The method of claim 23 wherein the level of
secretion TNF α , IL12P70 and IL10 is measured.

10 25. A method as claimed in any one of claims 15
to 24 wherein said immune stimulus is
lipopolysaccharide, TNF α , CD40L or monocyte
conditioned medium (MCM).

15 26. A method as claimed in any one of claims 15
to 25 wherein if said test molecule is found to be a
potential agonist of CD36 and/or CD51 the method
further comprises the step of exposing said molecule
20 to a purified sample of CD36 and/or CD51 and
detecting any direct binding between said molecule
and CD36 and/or CD51.

25 27. A method as claimed in claim 26 wherein said
purified CD36 or CD51 is immobilised to a solid
surface.

28. A method as claimed in claim 26 or claim 27
wherein said molecule is labelled with a detectable
30 label.

29. A method as claimed in any of claims 26 to
28 which further comprises the step of exposing said
molecule to a purified sample of $\alpha_v\beta_3$ or $\alpha_v\beta_5$ and
35 detecting any direct binding between said molecule and
said $\alpha_v\beta_3$ or $\alpha_v\beta_5$.

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30. A method as claimed in any one of claims 26 to 29 which further comprises the step of exposing said molecule to a purified sample of thrombospondin and detecting any direct binding between said molecule and thrombospondin.

31. A method as claimed in claim 29 or claim 30 wherein said molecule is labelled with a detectable label.

32. A method as claimed in any of claims 29 to 31 wherein said $\alpha_v\beta_3$, $\alpha_v\beta_5$ or thrombospondin is immobilised to a solid surface.

33. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of the cell surface receptor CD36 as expressed on mammalian dendritic cells and a pharmacologically acceptable carrier or diluent.

34. A composition as claimed in claim 33 which is suitable for inducing peripheral immune tolerance in a human wherein said CD36 agonist is selected from: an antibody with an affinity for an epitope of CD36, the Plasmodium falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1 for CD36 and/or thrombospondin, thrombospondin, apoptotic cells or a negatively-charged phospholipid.

35. A composition as claimed in claim 20 wherein said CD36 agonist is any one of the antibodies listed in Appendix 1.

36. A composition as claimed in claim 20 wherein the pf-EMP-1 active binding domain comprises the amino acid sequence as shown in Figure 2.

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37. A composition as claimed in claim 19 wherein said CD36 agonist is a molecule identified as such by any one of the methods of claims 15 to 32.

5 38. An agonist of the cell surface receptor CD36 as expressed on mammalian dendritic cells for use as a medicament.

10 39. An agonist for use as claimed in claim 38 wherein said medicament is used to induce a state of immune tolerance in a human.

15 40. An agonist for use as claimed in claim 38 or 39 which is suitable for treating a human and wherein said CD36 agonist is selected from: an antibody with an affinity for an epitope of CD36, the Plasmodium falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1 for CD36 or thrombospondin, thrombospondin, apoptotic cells or a
20 negatively charged phospholipid.

25 41. An agonist for use as claimed in claim 40 wherein said CD36 agonist is any one of the antibodies listed in Appendix 1.

 42. An agonist for use as claimed in claim 40 wherein the pf-EMP-1 active binding domain comprises the amino acid sequence as shown in Figure 2.

30 43. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of the cell surface receptor CD51 as expressed by mammalian dendritic cells and a pharmacologically acceptable carrier or diluent.

35 44. A composition as claimed in claim 43 suitable for inducing immune tolerance in a human

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wherein said CD51 agonist is selected from: an antibody with an affinity for an epitope of CD51, thrombospondin, apoptotic cells or a negatively charged phospholipid.

5

45. A composition as claimed in claim 44 wherein said CD51 agonist is any one of the antibodies listed in Appendix 2.

10

46. A composition as claimed in claim 43 or 44 which comprises the Plasmodium falciparum protein pf-EMP-1 or a protein comprising an active binding domain thereof and thrombospondin.

15

47. A composition as claimed in claim 46 wherein said active binding domain of pf-EMP-1 comprises the amino acid sequence shown in Figure 2.

20

48. A composition as claimed in claim 43 wherein said CD51 agonist is a molecule identified as such by any one of the methods of claims 15 to 32.

25

49. An agonist of the cell surface receptor CD51 as expressed on mammalian dendritic cells for use as a medicament.

30

50. An agonist for use as claimed in claim 49 wherein said medicament is used to induce a state of immune tolerance in a human.

35

51. An agonist for use as claimed in claim 49 or 50 which is suitable for administration to a human wherein said CD 51 agonist is selected from: an antibody with an affinity for an epitope of CD51, thrombospondin, apoptotic cells or a negatively charged phospholipid.

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52. An agonist for use as claimed in claim 51 wherein said CD51 agonist is any one of the antibodies listed in Appendix 2.

5 53. An agonist for use as claimed in claim 49 which is suitable for administration to a human and which comprises, in combination, the Plasmodium falciparum protein pf-EMP-1 or a protein comprising an active binding domain thereof and thrombospondin.

10 54. Use of a method comprising the following steps for identifying a molecule capable of preventing the adherence of red blood cells infected with a malarial parasite to human dendritic cells:

15 a) exposing a purified preparation of the human cell surface receptor CD36 to:-

 I) the molecule to be tested and
 ii) parasitised human red blood cells,
either consecutively or simultaneously and

20 b) determining the level of adherence of said parasitised red blood cells to CD36

 wherein a reduction in the level of adherence in the presence of the test molecule compared to the level in the absence of said molecule is an
25 indication that said molecule is capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells.

 55. Use of a method comprising the following steps for identifying a molecule capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells:

30 a) exposing a purified preparation of human thrombospondin to:

35 I) the molecule to be tested and
 ii) parasitised human red blood cells,
either consecutively or simultaneously and

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b) determining the level of adherence of said parasitised red blood cells to thrombospondin, wherein a reduction in the level of adherence to thrombospondin in the presence of the test molecule compared to the level in the absence of said molecule is an indication that said molecule is capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells.

56. Use of a method as claimed in claim 54 or claim 55 wherein said red blood cells are infected with *Plasmodium falciparum*.

57. Use of a method as claimed in claim 56 wherein the *Plasmodium falciparum* strain is IT0/A4, IT0/C24 or MC.

58. Use of a method as claimed in claim 54 wherein said CD36 is immobilised on a solid surface.

59. Use of a method as claimed in claim 55 wherein said thrombospondin is immobilised on a solid surface.

60. Use of a method as claimed in claim 58 or claim 59 wherein the level of adherence of said parasitised red blood cells to CD36 or thrombospondin is determined by the additional steps of:

a) washing the immobilised CD36 or thrombospondin to remove non-adhered red blood cells and

b) applying a stain to said immobilised CD36 or thrombospondin which is specific for parasitised or non-parasitised red blood cells.

61. Use of a method as claimed in claim 60 wherein said stain is detectable by eye, by microscopy or by a spectrophotometric method.

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62. Use of a method as claimed in claim 54 which comprises applying simultaneously or consecutively the method of claim 55.

5

63. A method of identifying a molecule capable of preventing the adherence of red blood cells infected with a malarial parasite to human dendritic cells which comprises:

10 a) exposing immature human dendritic cells to the Plasmodium falciparum protein pf-EMP-1 or an active binding domain thereof in the presence or absence of the molecule to be tested,

15 b) exposing said immature dendritic cells to an immune stimulus and

c) determining the degree of maturation manifested by said dendritic cells,

20 wherein any maturation of said dendritic cells in the presence of the test molecule over and above that manifested in the absence of said molecule is an indication that said molecule is capable of preventing adherence of red blood cells infected with a malarial parasite to human dendritic cells.

25 64. A method as claimed in claim 63 wherein maturation of said dendritic cells is determined by examining the antigen-presenting ability of said cells.

30 65. A method as claimed in claim 63 or claim 64 wherein maturation of said dendritic cells is determined by examining said cells for expression of at least one cell surface antigen whose expression level is enhanced in response to an immune stimulus.

35

66. A method as claimed in claim 65 wherein maturation of said dendritic cells is determined by

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measuring the level of expression of two or more of the following panel of antigens:
HLA DR, CD54, CD40, CD83 and CD86.

5 67. A method as claimed in claim 66 wherein said cells are also examined for expression of CD80.

68. A method as claimed in any one of claims 65 to 67 wherein the level of expression of said antigen
10 is detected using a labelled antibody.

69. A method as claimed in claim 63 wherein maturation is determined by measuring said cells' ability to induce T-cell proliferation.
15

70. A method as claimed in claim 63 wherein maturation of said dendritic cells is determined by quantifying the level of cytokines secreted from said cells.
20

71. A method as claimed in claim 70 wherein the level of secretion of TNF α , IL12P70 and IL10 is measured.

25 72. A method as claimed in any one of claims 63 to 71 wherein said immune stimulus is lipopolysaccharide, TNF alpha, CD40L or monocyte conditioned medium (MCM).

30 73. A pharmaceutical composition useful for the treatment of malaria which comprises a molecule capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells which has been identified by the method of any
35 one of claims 63 to 72 and a pharmacologically acceptable carrier or diluent.

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74. A molecule identified as being capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells by the method of any one of claims 63 to 72 for use in the treatment of malaria.

75. A pharmaceutical composition useful for the treatment of malaria which comprises a molecule capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells which has been identified by the use of the method of any one of claims 54 to 62.

76. A molecule identified as being capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells by use of the method as claimed in any one of claims 54 to 62.

77. A method of identifying a molecule which is an agonist of cell surface receptors CD36 and/or CD51 and/or a receptor for thrombospondin as expressed on antigen-presenting cells of the mammalian immune system which method comprises:

a) exposing mammalian antigen-presenting cells to the molecule to be tested,
b) exposing said cells to an immune stimulus and
c) determining the response to said immune stimulus by said cells,

wherein an impaired response compared to the response in the absence of said test molecule is an indication that said molecule under test is a agonist of CD36 and/or CD51 and/or a thrombospondin receptor.

78. A method as claimed in claim 77 wherein said thrombospondin receptor is not CD47.

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79. A method as claimed in claim 77 or claim 78 wherein said response is maturation of said antigen-presenting cell.

5 80. A method as claimed in any one of claims 77 to 79 wherein said antigen-presenting cell of the immune system is selected from a dendritic cell, a macrophage, a B-lymphocyte or a monocyte.

10 81. A method as claimed in any of claims 77 to 80 which includes the features of any of claims 16 to 18 or 21 to 32.

15 82. A method of treating mammalian antigen-presenting cells in vitro to induce immune tolerance therein which comprises exposing said cells to an agonist of the cell surface receptor for thrombospondin and/or an agonist for the cell surface receptors CD36 and/or CD51.

20 83. A method as claimed in claim 82 wherein said antigen-presenting cell is human or mouse.

25 84. A method as claimed in claim 82 or 83 wherein said antigen presenting cell is selected from a dendritic cell, a macrophage, a B-lymphocyte or a monocyte.

30 85. A method as claimed in any one of claims 82 to 84 wherein said agonist is a molecule identified as such by the method of any one of claims 77 to 81.

35 86. A method as claimed in any one of claims 82 to 85 which includes the features of any one of claims 4 to 12.

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87. A method as claimed in any one of claims 82 to 85 wherein said antigen-presenting cells are exposed to an antibody to an epitope of a thrombospondin receptor.

5

88. A method as claimed in claim 87 wherein said agonist is any one of the antibodies listed in Appendix 3.

10

89. A method as claimed in claim 87 or claim 88, which includes the features of any of claims 9 to 12.

15

90. An antigen-presenting cell preparation obtainable by the method of any one of claims 82 to 89 for use as a medicament.

20

91. As antigen-presenting cell preparation obtainable by the method of any one of claims 82 to 89 for use in inducing peripheral immune tolerance in a human.

25

92. A method of identifying a molecule which is an agonist of a β -integrin associated with the cell surface receptor CD51 as expressed on antigen-presenting cells of the mammalian immune system which method comprises:

30

- a) exposing mammalian antigen-presenting cells to the molecule to be tested,
- b) exposing said cells to an immune stimulus and
- c) determining the response to said immune stimulus by said cells,

35

wherein an impaired response compared to the response in the absence of said test molecule is an indication that said molecule is an agonist of a β -integrin associated with the cell surface receptor CD51.

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93. A method as claimed in claim 92 which includes the features of claim 78 or claim 79

5 94. A method as claimed in claim 92 to 93 which further includes the features of any one of claims 16 to 25 or 29 to 32.

10 95. A method as claimed in any one of claims 92 to 94 wherein said β -integrin is $\beta 3$ or $\beta 5$.

15 96. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of a β -integrin associated with the cell surface receptor CD51 as expressed on mammalian antigen-presenting cells and a pharmacologically acceptable carrier or diluent.

20 97. A pharmaceutical composition as claimed in claim 96 wherein the β -integrin is $\beta 3$ or $\beta 5$.

25 98. A method of inducing a state of immune tolerance in antigen-presenting cells of the mammalian immune system which comprises exposing said cells ex-vivo to an agonist of a β -integrin.

99. A method as claimed in claim 97 wherein said β -integrin is $\beta 3$ or $\beta 5$.

30 100. A method as claimed in claim 97 to 98 wherein said antigen presenting cells are human.

101. A method as claimed in claim 97 or 99 wherein said cells are exposed to an antigenic material.

35 102. A preparation of cells obtainable by the method as claimed in any one of claims 97 to 100 for

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use as a medicament.

103. A preparation of cells obtainable by the
method as claimed in any one of claims 97 to 100 for
5 use in inducing immune-tolerance in a human.

104. A pharmaceutical composition suitable for
inducing peripheral immune tolerance in a mammal which
comprises an agonist of a thrombospondin receptor and
10 a pharmacologically acceptable carrier or diluent.

105. A composition as claimed in claim 104
wherein said thrombospondin receptor is not CD47.

15 106. A preparation of apoptotic cells for use in
inducing peripheral immune tolerance in a mammal.

107. A preparation as claimed in claim 106
wherein said mammal is a human.
20

108. A preparation comprising a negatively
charged phospholipid for use in inducing peripheral
immune tolerance in a mammal.

25 109. A preparation as claimed in claim 108
wherein said mammal is a human.

110. A preparation as claimed in claim 108 or 109
which comprises liposomes including a negatively
30 charged phospholipid.

111. A preparation as claimed any one of claims
108 to 110 wherein said negatively charged
phospholipid is phosphatidylserine.
35

112. A method of inducing a state of immune
tolerance in antigen-presenting cells of the mammalian

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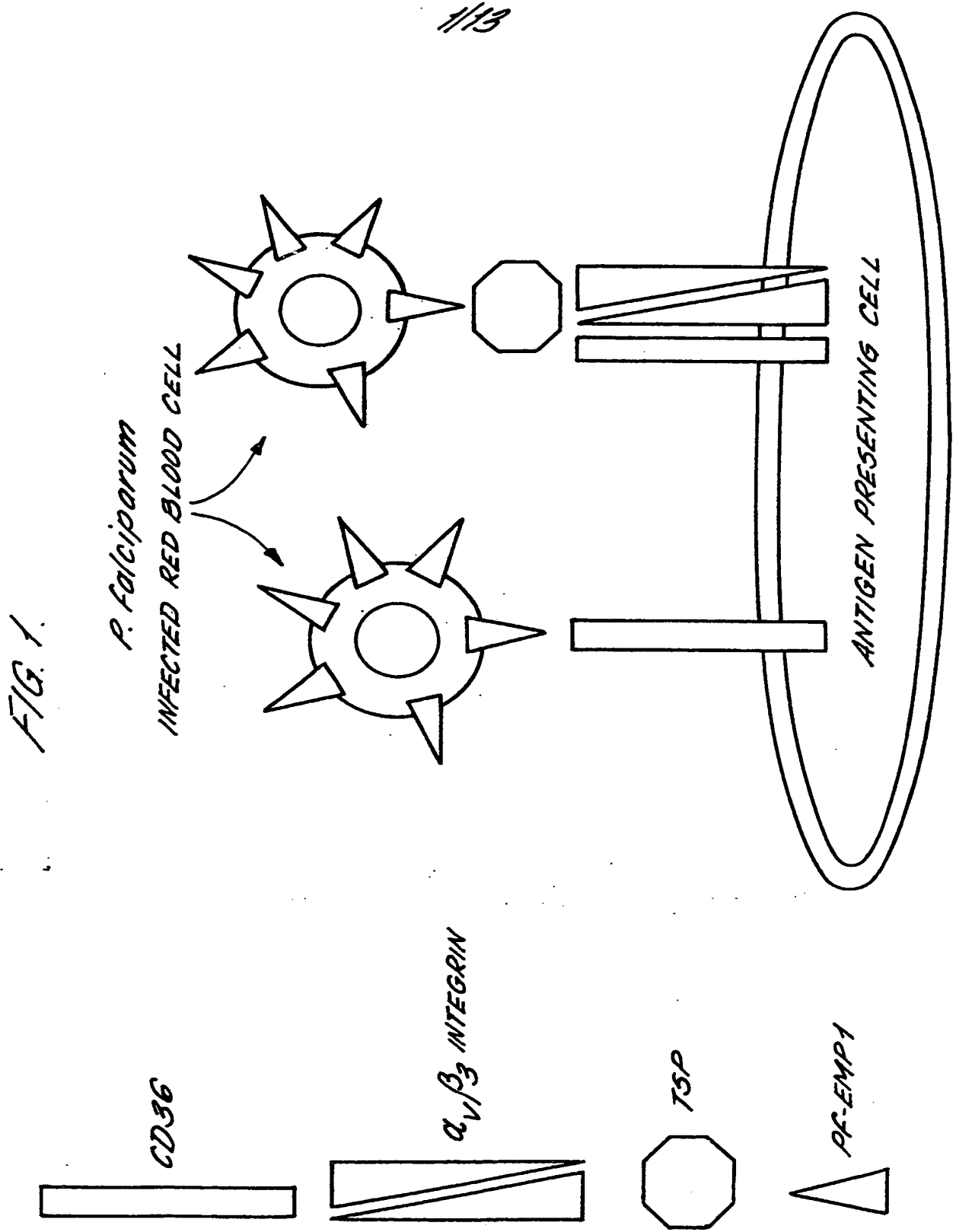
immune system which comprises exposing said cells *ex-vivo* to a composition or preparation as claimed in any one of claims 104 to 111.

5 113. A method as claimed in claim 112 wherein said cells are exposed to an antigenic material.

114. A preparation of antigen-presenting cells obtainable by the method of claims 112 or 113.

10

115. A method of treating a human to induce peripheral immune tolerance therein comprising administering to said human a substance selected from the group consisting of: an agonist of CD36, an
15 agonist of CD51, an agonist of a thrombospondin receptor, an agonist of a β -integrin and a preparation of cells of any of claims 13, 14, 90, 91, 102 or 114.



NOT RECD [REDACTED] I/PTC 21 DEC 2001

2/13

FIG. 2.

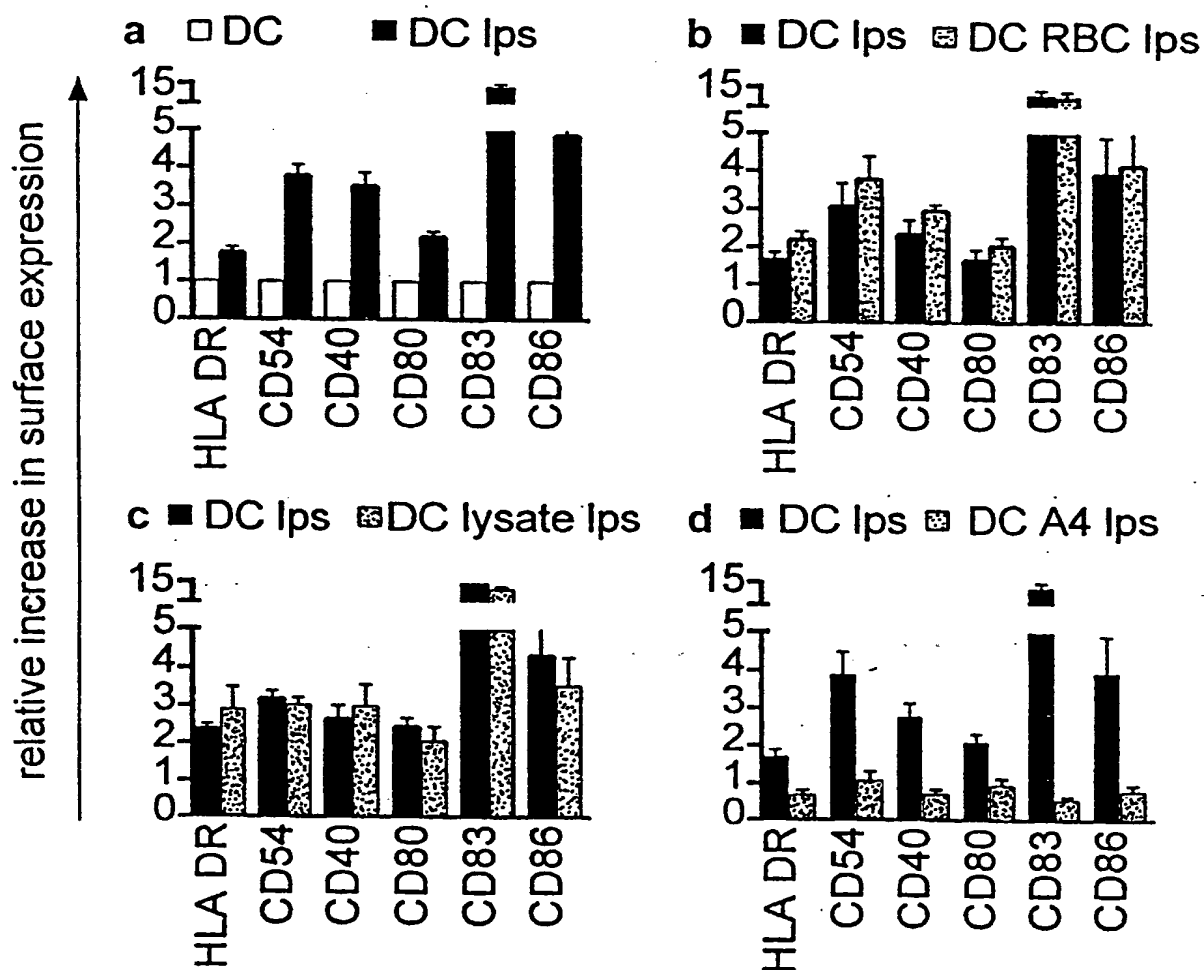
**AMINO ACID SEQUENCE OF THE CIDR FRAGMENT OF THE A4-VAR
GENE (COMPLETE SEQUENCE ACCESSION NO L42244)**

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REPCPWCIE EQKDGKWKRI NDHSACEEEE LYTPKENAKY
TKINVLTSGE GHEDIAKRLK EFCTKTQNGG GGSDDCGGNS
DSSLCEPWQC YQPDQLEKVG GGEVDDKLKG AGGLCIFEKM
KGEKKVKKQK TFNNEFNEWV AHVLKDSIDW RTQLTKCLSE
DKLKKCEKGC KSNCECFKKW IEKKEKEWIK VKDQFNKQTD
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DECVEEIEKI FNNPCSGTRH RAMVKNVAAD MYRAARQQLR
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IGDSKD -846

531 Rec'd PCIT: 21 DEC 2001

3/13

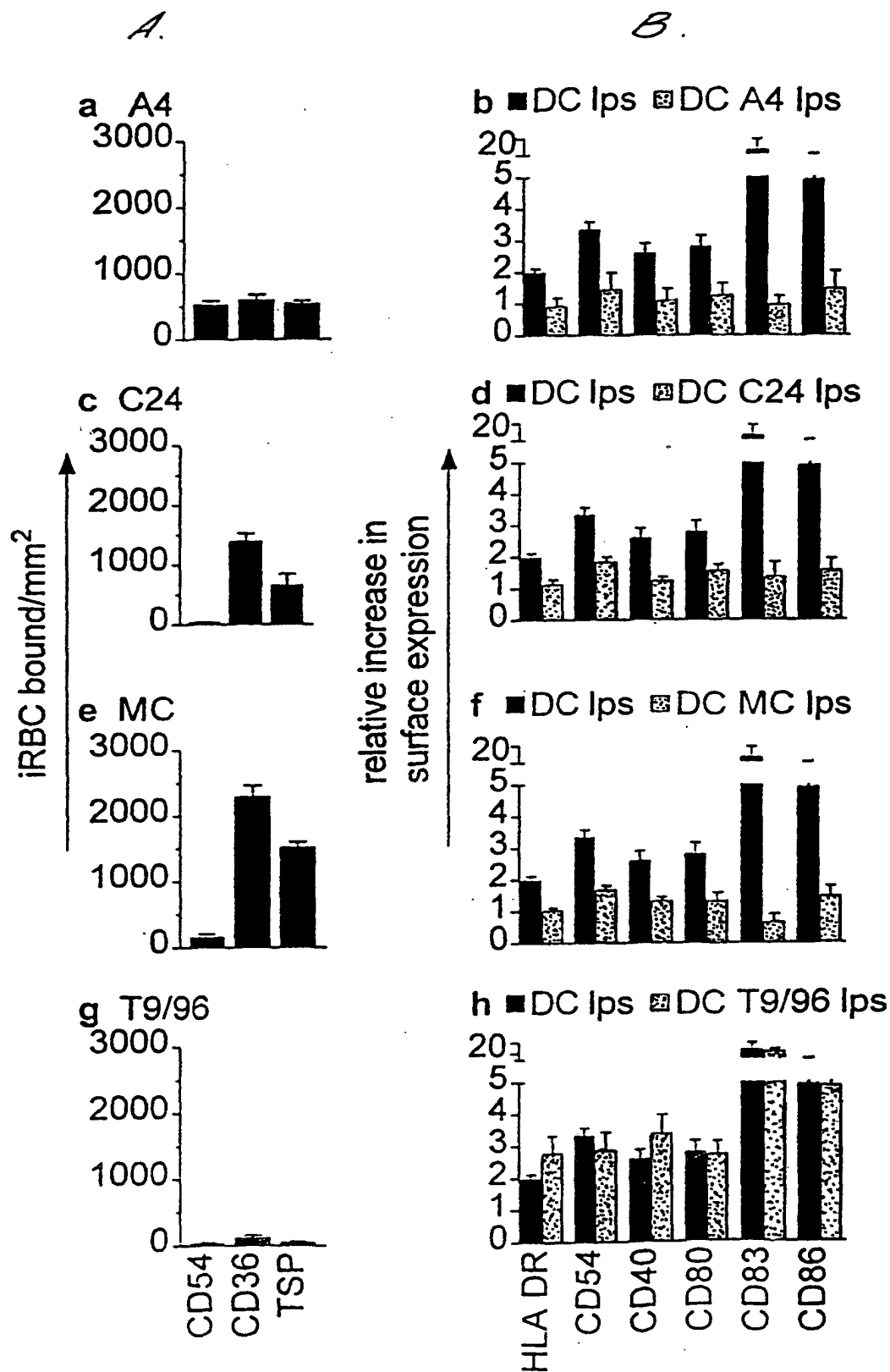
FIG. 3.



531 Rec'd PCH

1 DEC 2001

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FIG. 4.

531 Rec'd POF

21 DEC 2001

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FIG. 5.

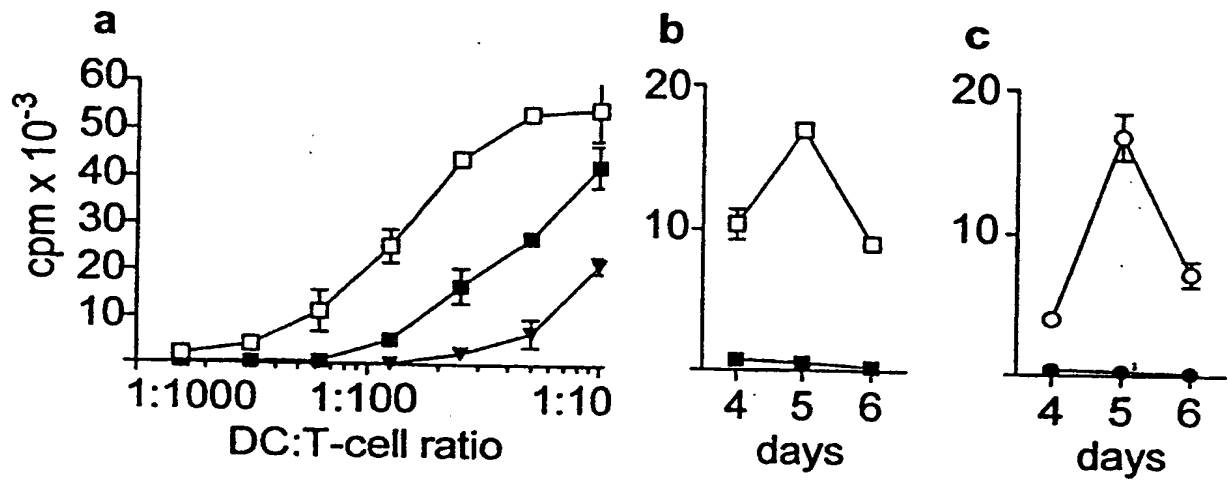


531 Rec'd PCT

21 DEC 2001

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FIG. 6.

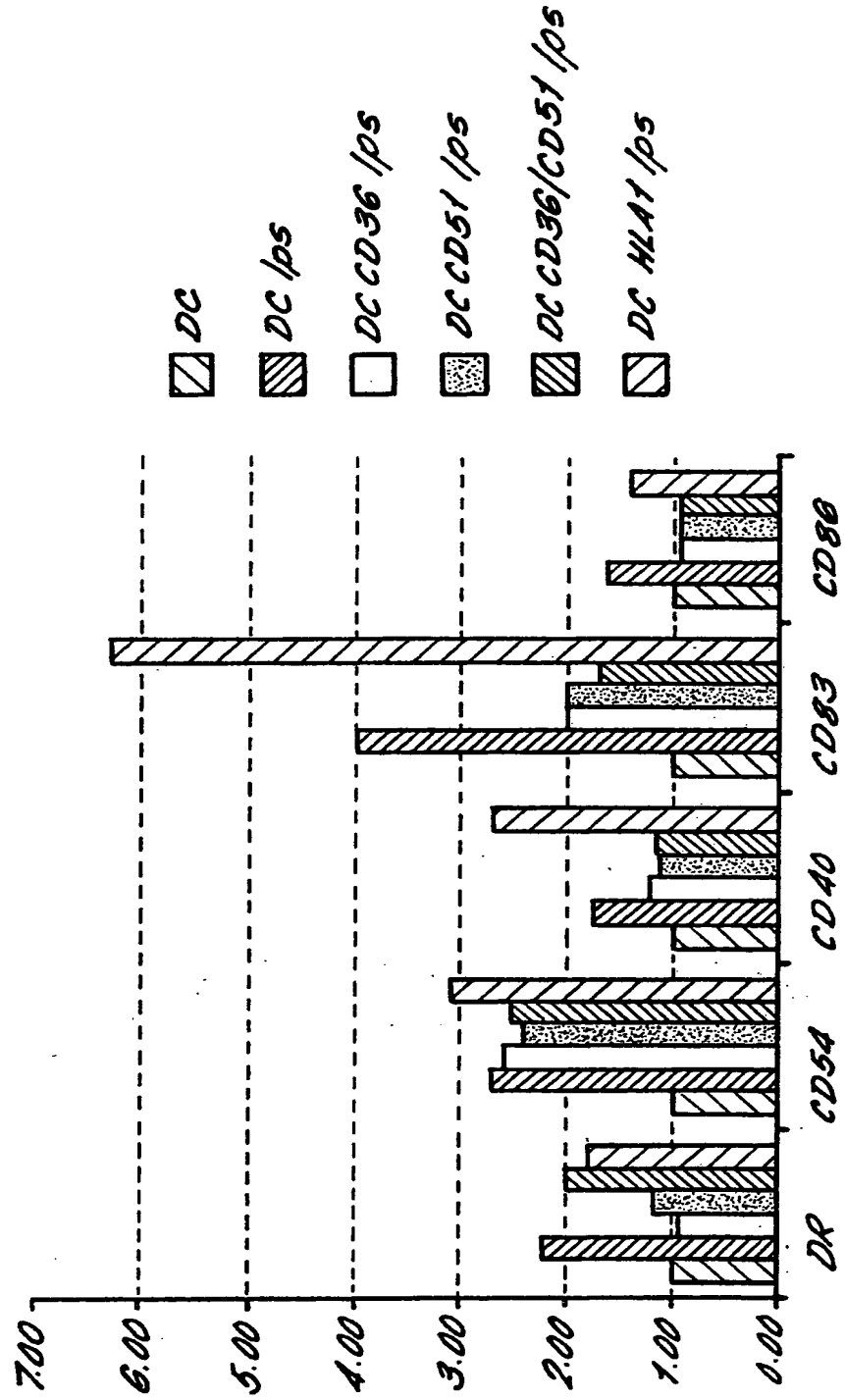


531 Rec'd PCT

21 DEC 2001

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FIG. 7



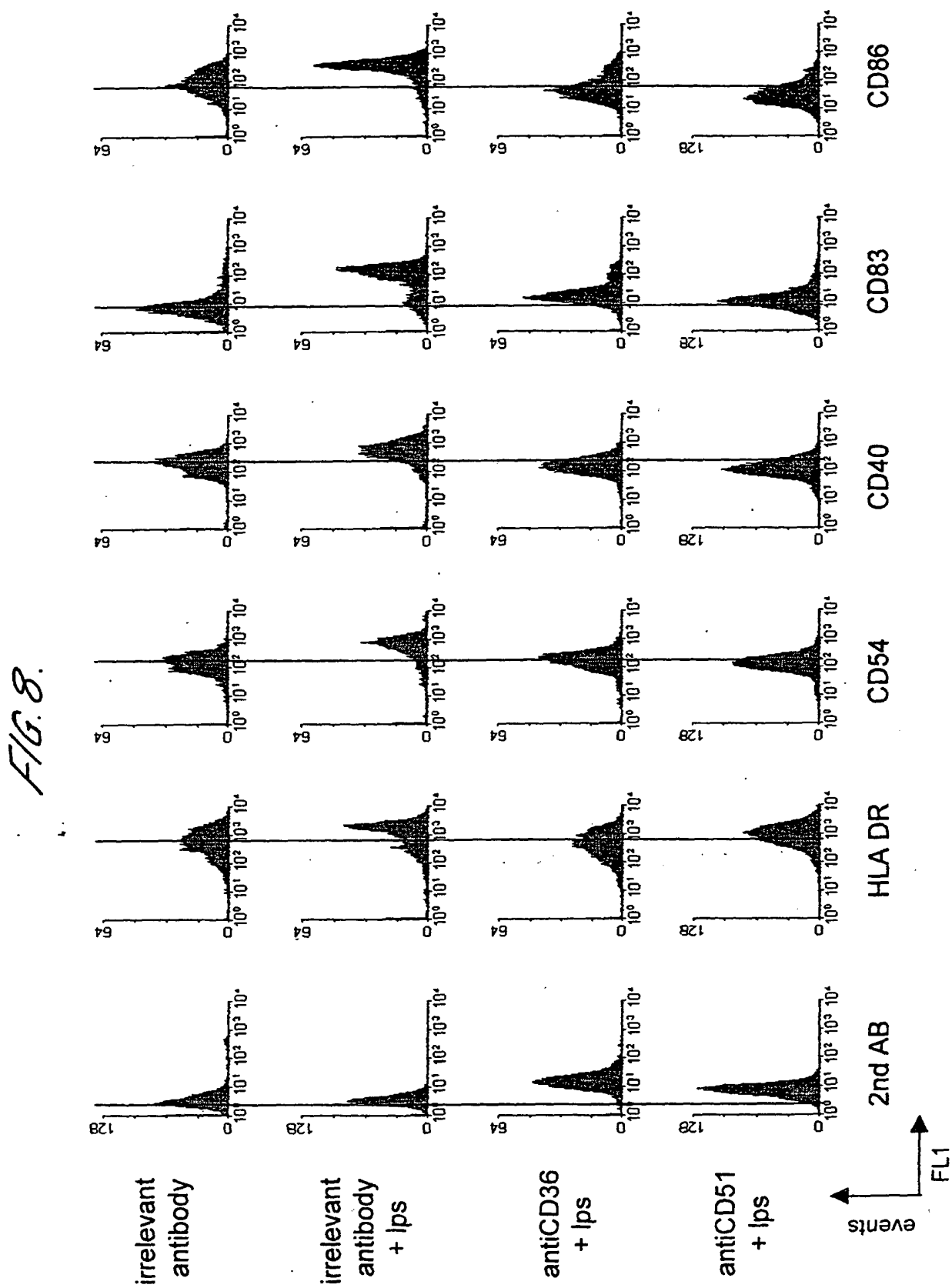
531 Rec'd P075



21 DEC 2001

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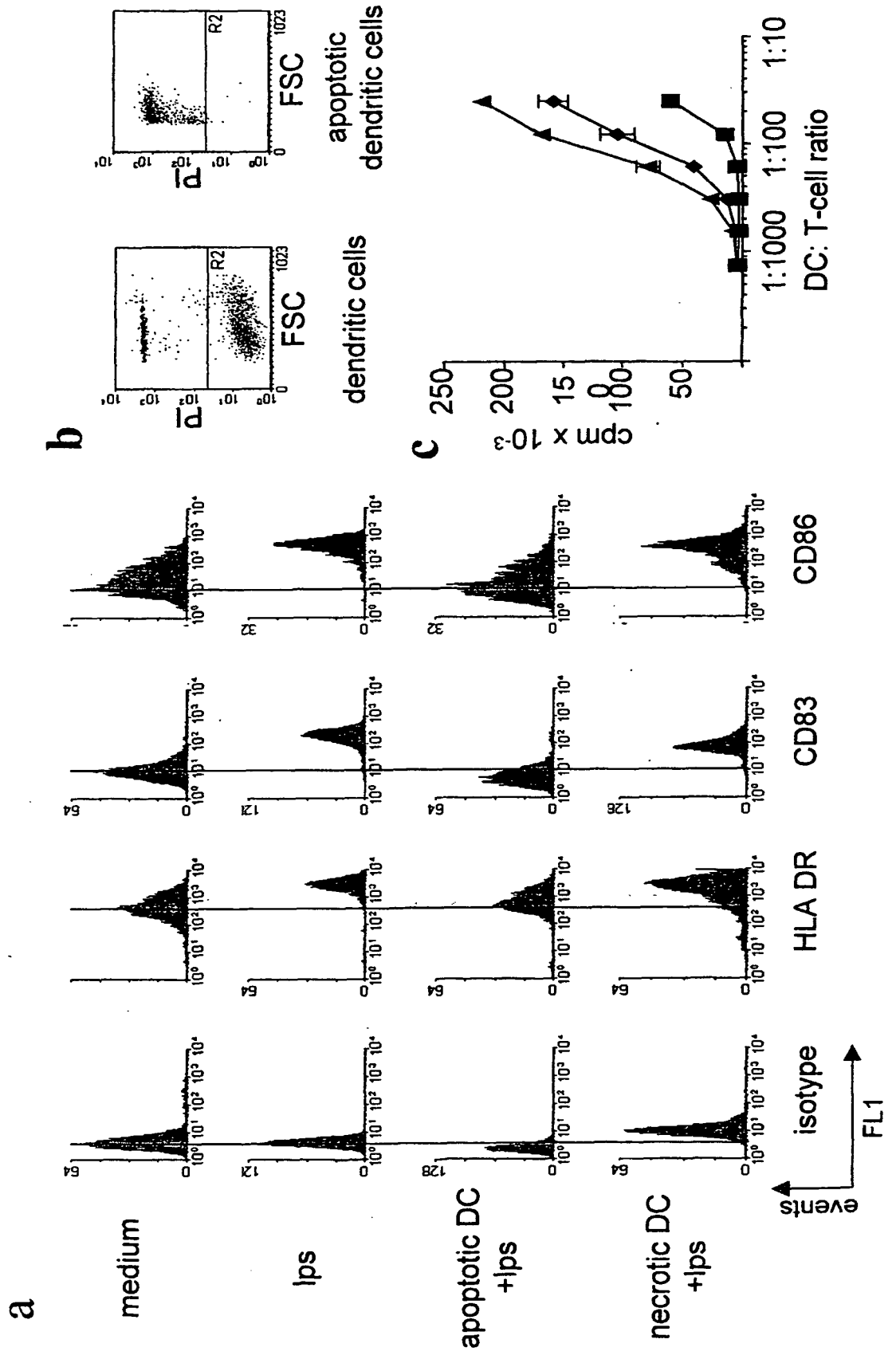


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21 DEC 2001

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FIG. 9.

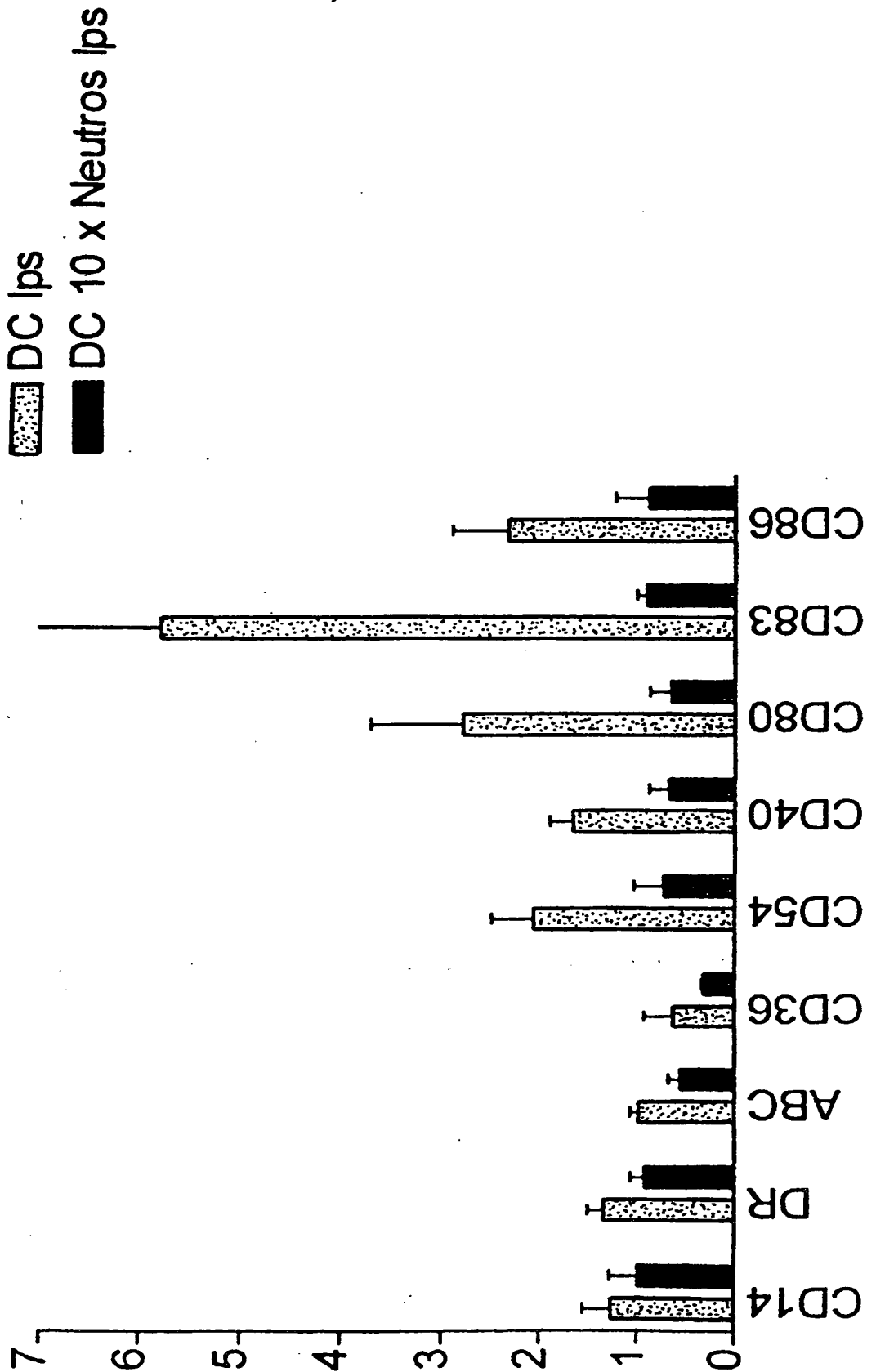


531 Rec'd PPTC 21 DEC 2001

10/019580

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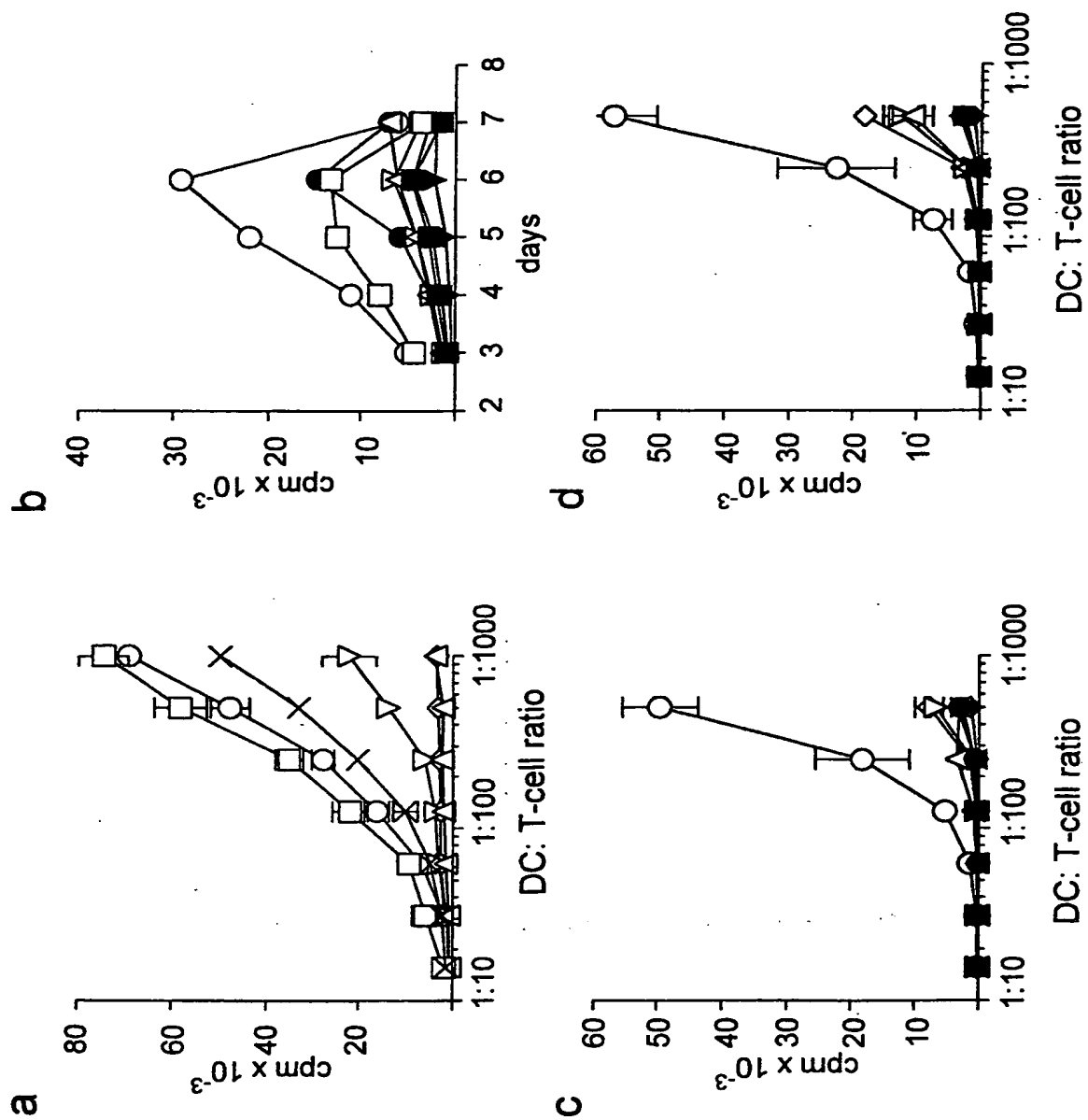
FIG. 10.



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FIG. 11.

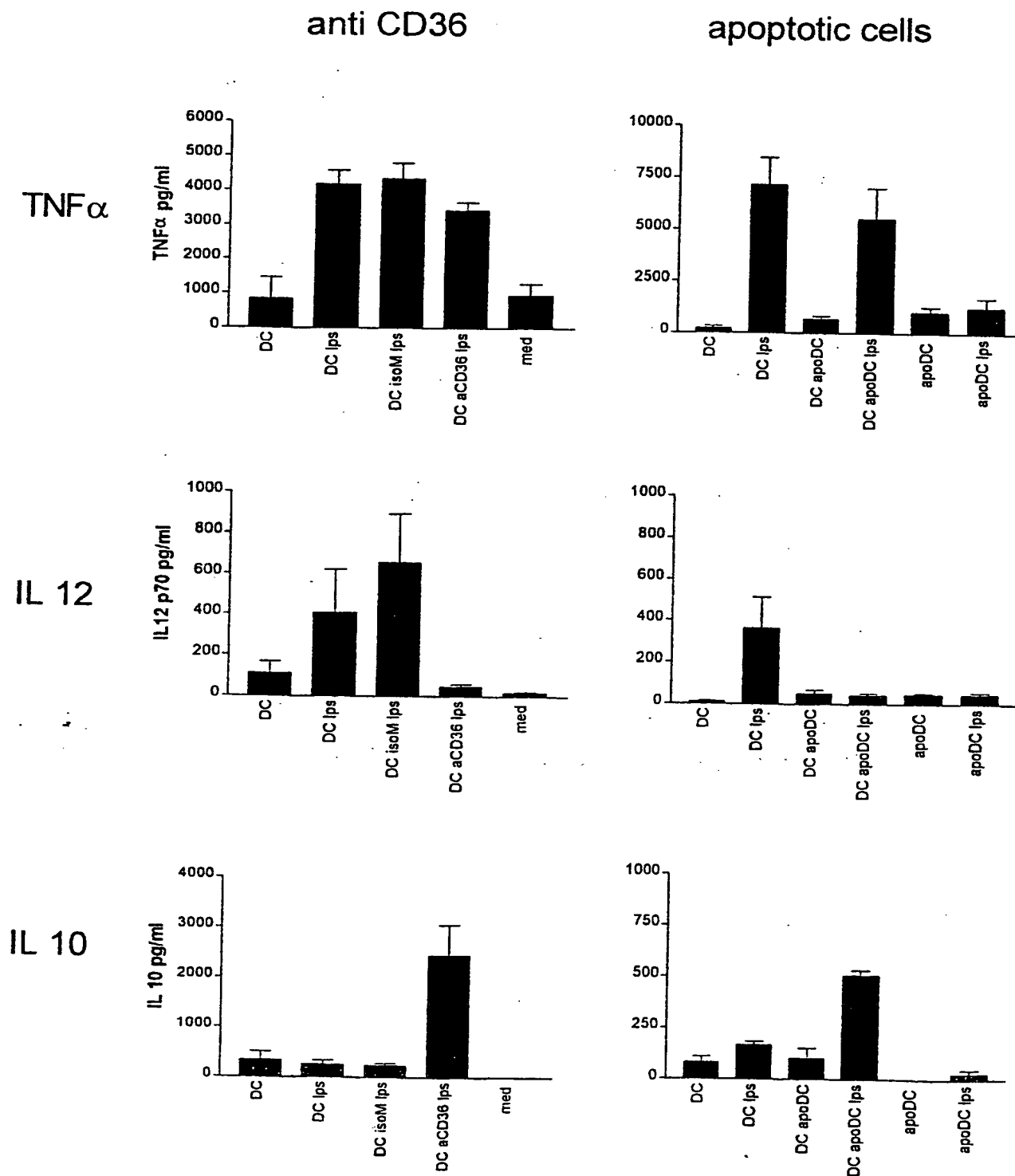


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FIG. 12.



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FIG. 13.

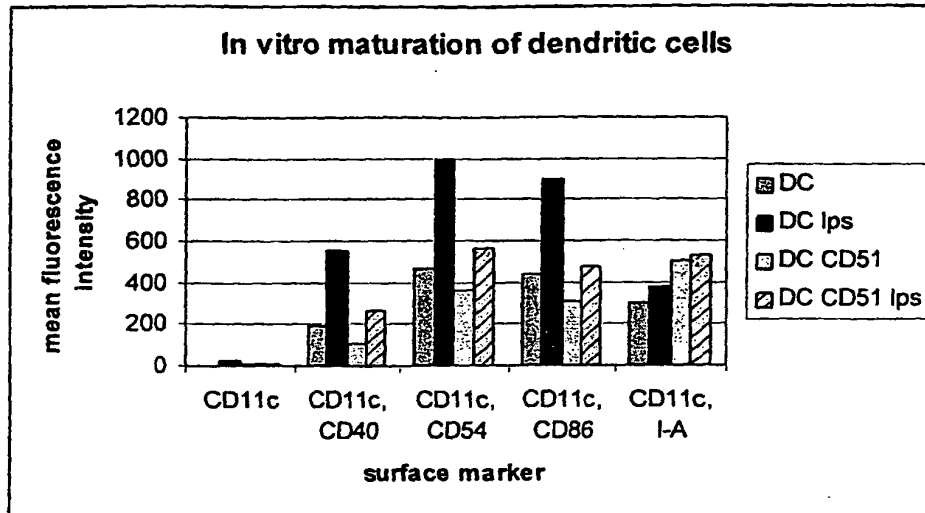
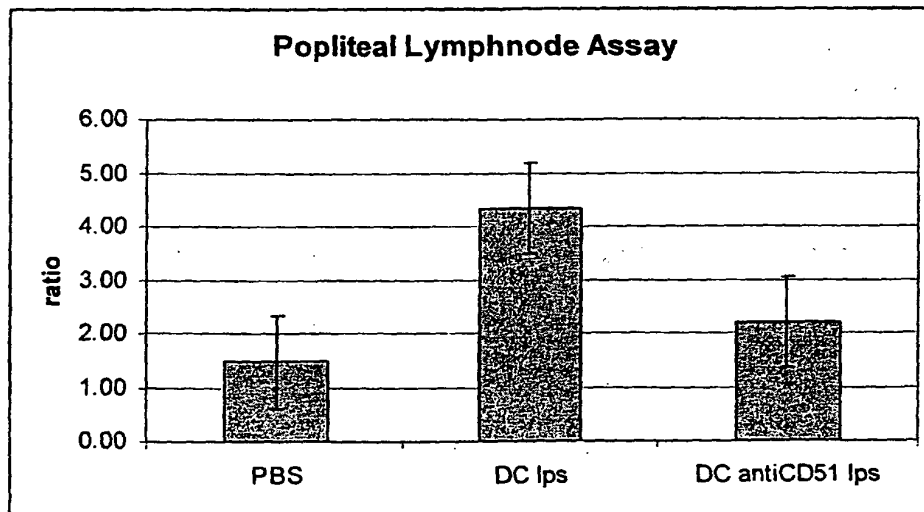


FIG. 14.



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CLAIMS:

1. A method of treating mammalian dendritic cells in vitro to induce immune tolerance therein which comprises exposing said cells to an agonist of the cell surface receptors CD36 and/or CD51 as expressed on mammalian dendritic cells.
2. A method as claimed in claim 1 wherein said dendritic cells are human or mouse.
3. A method as claimed in claim 1 or 2 wherein said agonist is a molecule identified as such by any one of the methods of claims 15 to 32.
4. A method as claimed in claim 1 or 2 wherein said agonist is selected from: an antibody with an affinity for an epitope of CD36, an antibody with an affinity for an epitope of CD51, the Plasmodium falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1, thrombospondin, apoptotic cells or a negatively charged phospholipid.
5. A method as claimed in claim 1 or 2 which comprises exposing said dendritic cells to two or more of the agonists of claim 4.
6. A method as claimed in claim 4 or claim 5 wherein the CD36 agonist any one of the antibodies listed in Appendix 1.
7. A method as claimed in claim 4 or claim 5 wherein the CD51 agonist is any one of the antibodies listed in Appendix 2.
8. A method as claimed in claim 4 or claim 5

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wherein the pf-EMP-1 active binding domain comprises the amino acid sequence shown in Figure 2.

5 9. A method as claimed in any one of claims 40 to 47 wherein said cells are exposed to an antigenic material.

10 10. A method as claimed in claim 9 wherein said antigenic material is an auto-antigen associated with a particular auto-immune disease.

15 11. A method as claimed in claim 9 or 10 wherein the cells so produced are subsequently matured by exposure to an immune stimulus.

20 12. A method as claimed in any of claims 1 to 11 wherein said dendritic cells are prepared from human peripheral blood and or derived from CD34+ stem cells or monocytes.

25 13. A dendritic cell preparation obtainable by the method of any of claims 1 to 12 for use as a medicament.

30 14. A dendritic cell preparation obtainable by the method of any of claims 1 to 12 for use in inducing peripheral immune tolerance in a human.

35 15. A method of identifying a molecule which is an agonist of cell surface receptor CD36 and/or CD51 as expressed by mammalian dendritic cells which method comprises:

- a) exposing immature mammalian dendritic cells to the molecule to be tested,
- 35 b) exposing said immature dendritic cells to an immune stimulus and

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c) determining the degree of maturation
manifested by said dendritic cells,

wherein impaired maturation in response to the
immune stimulus is an indication that said molecule
5 under test is a CD36 and/or CD51 agonist.

16. A method as claimed in claim 15 wherein
said dendritic cells are human cells or mouse cells.

10 17. A method as claimed in claim 15 or 16
wherein maturation of said dendritic cells is
determined by examining the antigen-presenting ability
of said cells.

15 18. A method as claimed in any of claims 15 to
17 wherein maturation of said dendritic cells is
determined by examining said cells for expression of
at least one cell surface antigen whose level of
expression is enhanced in response to an immune
20 stimulus.

19. A method as claimed in claim 18 wherein
maturation of said dendritic cells is determined by
measuring the level of expression of one or more of
25 the following panel of antigens:
HLA DR, CD54, CD40, CD83 and CD86.

20. A method as claimed in claim 19 wherein
said cells are also examined for expression of CD80.

30 21. A method as claimed in any one of claims 18
to 20 wherein the level of expression of said antigens
is detected using a labelled antibody.

35 22. A method as claimed in 15 or 16 wherein
maturation of said dendritic cells is determined by
measuring said cells' ability to induce T-cell

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proliferation.

23. The method of claim 15 or 16 wherein
maturation of said dendritic cells is determined by
5 quantifying the level of cytokines secreted from said
cells.

24. The method of claim 23 wherein the level of
secretion TNF α , IL12P70 and IL10 is measured.
10

25. A method as claimed in any one of claims 15
to 24 wherein said immune stimulus is
lipopolysaccharide, TNF α , CD40L or monocyte
conditioned medium (MCM).
15

26. A method as claimed in any one of claims 15
to 25 wherein if said test molecule is found to be a
potential agonist of CD36 and/or CD51 the method
further comprises the step of exposing said molecule
20 to a purified sample of CD36 and/or CD51 and
detecting any direct binding between said molecule
and CD36 and/or CD51.

27. A method as claimed in claim 26 wherein said
25 purified CD36 or CD51 is immobilised to a solid
surface.

28. A method as claimed in claim 26 or claim 27
wherein said molecule is labelled with a detectable
30 label.

29. A method as claimed in any of claims 26 to
28 which further comprises the step of exposing said
molecule to a purified sample of $\alpha_v\beta_3$ or $\alpha_v\beta_5$ and
35 detecting any direct binding between said molecule and
said $\alpha_v\beta_3$ or $\alpha_v\beta_5$.

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30. A method as claimed in any one of claims 26 to 29 which further comprises the step of exposing said molecule to a purified sample of thrombospondin and detecting any direct binding between said molecule and thrombospondin.

31. A method as claimed in claim 29 or claim 30 wherein said molecule is labelled with a detectable label.

32. A method as claimed in any of claims 29 to 31 wherein said $\alpha_v\beta_3$, $\alpha_v\beta_5$ or thrombospondin is immobilised to a solid surface.

33. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of the cell surface receptor CD36 as expressed on mammalian dendritic cells and a pharmacologically acceptable carrier or diluent.

34. A composition as claimed in claim 33 which is suitable for inducing peripheral immune tolerance in a human wherein said CD36 agonist is selected from: an antibody with an affinity for an epitope of CD36, the Plasmodium falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1 for CD36 and/or thrombospondin, thrombospondin, apoptotic cells or a negatively-charged phospholipid.

35. A composition as claimed in claim 20 wherein said CD36 agonist is any one of the antibodies listed in Appendix 1.

36. A composition as claimed in claim 20 wherein the pf-EMP-1 active binding domain comprises the amino acid sequence as shown in Figure 2.

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37. A composition as claimed in claim 19 wherein said CD36 agonist is a molecule identified as such by any one of the methods of claims 15 to 32.

5 38. An agonist of the cell surface receptor CD36 as expressed on mammalian dendritic cells for use as a medicament.

10 39. An agonist for use as claimed in claim 38 wherein said medicament is used to induce a state of immune tolerance in a human.

15 40. An agonist for use as claimed in claim 38 or 39 which is suitable for treating a human and wherein said CD36 agonist is selected from: an antibody with an affinity for an epitope of CD36, the Plasmodium falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1 for CD36 or thrombospondin, thrombospondin, apoptotic cells or a
20 negatively charged phospholipid.

 41. An agonist for use as claimed in claim 40 wherein said CD36 agonist is any one of the antibodies listed in Appendix 1.

25

 42. An agonist for use as claimed in claim 40 wherein the pf-EMP-1 active binding domain comprises the amino acid sequence as shown in Figure 2.

30 43. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of the cell surface receptor CD51 as expressed by mammalian dendritic cells and a pharmacologically acceptable carrier or diluent.

35

 44. A composition as claimed in claim 43 suitable for inducing immune tolerance in a human

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wherein said CD51 agonist is selected from: an antibody with an affinity for an epitope of CD51, thrombospondin, apoptotic cells or a negatively charged phospholipid.

5

45. A composition as claimed in claim 44 wherein said CD51 agonist is any one of the antibodies listed in Appendix 2.

10

46. A composition as claimed in claim 43 or 44 which comprises the Plasmodium falciparum protein pf-EMP-1 or a protein comprising an active binding domain thereof and thrombospondin.

15

47. A composition as claimed in claim 46 wherein said active binding domain of pf-EMP-1 comprises the amino acid sequence shown in Figure 2.

20

48. A composition as claimed in claim 43 wherein said CD51 agonist is a molecule identified as such by any one of the methods of claims 15 to 32.

25

49. An agonist of the cell surface receptor CD51 as expressed on mammalian dendritic cells for use as a medicament.

30

50. An agonist for use as claimed in claim 49 wherein said medicament is used to induce a state of immune tolerance in a human.

35

51. An agonist for use as claimed in claim 49 or 50 which is suitable for administration to a human wherein said CD 51 agonist is selected from: an antibody with an affinity for an epitope of CD51, thrombospondin, apoptotic cells or a negatively charged phospholipid.

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52. An agonist for use as claimed in claim 51 wherein said CD51 agonist is any one of the antibodies listed in Appendix 2.

5 53. An agonist for use as claimed in claim 49 which is suitable for administration to a human and which comprises, in combination, the Plasmodium falciparum protein pf-EMP-1 or a protein comprising an active binding domain thereof and thrombospondin.

10 54. Use of a method comprising the following steps for identifying a molecule capable of preventing the adherence of red blood cells infected with a malarial parasite to human dendritic cells:

15 a) exposing a purified preparation of the human cell surface receptor CD36 to:-

I) the molecule to be tested and

ii) parasitised human red blood cells, either consecutively or simultaneously and

20 b) determining the level of adherence of said parasitised red blood cells to CD36

wherein a reduction in the level of adherence in the presence of the test molecule compared to the level in the absence of said molecule is an indication that said molecule is capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells.

30 55. Use of a method comprising the following steps for identifying a molecule capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells:

a) exposing a purified preparation of human thrombospondin to:

35 I) the molecule to be tested and

ii) parasitised human red blood cells, either consecutively or simultaneously and

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b) determining the level of adherence of said parasitised red blood cells to thrombospondin, wherein a reduction in the level of adherence to thrombospondin in the presence of the test molecule compared to the level in the absence of said molecule is an indication that said molecule is capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells.

56. Use of a method as claimed in claim 54 or claim 55 wherein said red blood cells are infected with *Plasmodium falciparum*.

57. Use of a method as claimed in claim 56 wherein the *Plasmodium falciparum* strain is ITO/A4, ITO/C24 or MC.

58. Use of a method as claimed in claim 54 wherein said CD36 is immobilised on a solid surface.

59. Use of a method as claimed in claim 55 wherein said thrombospondin is immobilised on a solid surface.

60. Use of a method as claimed in claim 58 or claim 59 wherein the level of adherence of said parasitised red blood cells to CD36 or thrombospondin is determined by the additional steps of:

a) washing the immobilised CD36 or thrombospondin to remove non-adhered red blood cells and

b) applying a stain to said immobilised CD36 or thrombospondin which is specific for parasitised or non-parasitised red blood cells.

61. Use of a method as claimed in claim 60 wherein said stain is detectable by eye, by microscopy or by a spectrophotometric method.

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62. Use of a method as claimed in claim 54 which comprises applying simultaneously or consecutively the method of claim 55.

5

63. A method of identifying a molecule capable of preventing the adherence of red blood cells infected with a malarial parasite to human dendritic cells which comprises:

10 a) exposing immature human dendritic cells to the Plasmodium falciparum protein pf-EMP-1 or an active binding domain thereof in the presence or absence of the molecule to be tested,

15 b) exposing said immature dendritic cells to an immune stimulus and

c) determining the degree of maturation manifested by said dendritic cells,

20 wherein any maturation of said dendritic cells in the presence of the test molecule over and above that manifested in the absence of said molecule is an indication that said molecule is capable of preventing adherence of red blood cells infected with a malarial parasite to human dendritic cells.

25 64. A method as claimed in claim 63 wherein maturation of said dendritic cells is determined by examining the antigen-presenting ability of said cells.

30 65. A method as claimed in claim 63 or claim 64 wherein maturation of said dendritic cells is determined by examining said cells for expression of at least one cell surface antigen whose expression level is enhanced in response to an immune stimulus.

35

66. A method as claimed in claim 65 wherein maturation of said dendritic cells is determined by

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measuring the level of expression of two or more of the following panel of antigens:

HLA DR, CD54, CD40, CD83 and CD86.

5 67. A method as claimed in claim 66 wherein said cells are also examined for expression of CD80.

10 68. A method as claimed in any one of claims 65 to 67 wherein the level of expression of said antigen is detected using a labelled antibody.

15 69. A method as claimed in claim 63 wherein maturation is determined by measuring said cells' ability to induce T-cell proliferation.

20 70. A method as claimed in claim 63 wherein maturation of said dendritic cells is determined by quantifying the level of cytokines secreted from said cells.

25 71. A method as claimed in claim 70 wherein the level of secretion of TNF α , IL12P70 and IL10 is measured.

30 72. A method as claimed in any one of claims 63 to 71 wherein said immune stimulus is lipopolysaccharide, TNF alpha, CD40L or monocyte conditioned medium (MCM).

35 73. A pharmaceutical composition useful for the treatment of malaria which comprises a molecule capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells which has been identified by the method of any one of claims 63 to 72 and a pharmacologically acceptable carrier or diluent.

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74. A molecule identified as being capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells by the method of any one of claims 63 to 72 for use in the treatment of malaria.

75. A pharmaceutical composition useful for the treatment of malaria which comprises a molecule capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells which has been identified by the use of the method of any one of claims 54 to 62.

76. A molecule identified as being capable of preventing the adherence of red blood cells infected with the malarial parasite to human dendritic cells by use of the method as claimed in any one of claims 54 to 62.

77. A method of identifying a molecule which is an agonist of cell surface receptors CD36 and/or CD51 and/or a receptor for thrombospondin as expressed on antigen-presenting cells of the mammalian immune system which method comprises:

a) exposing mammalian antigen-presenting cells to the molecule to be tested,
b) exposing said cells to an immune stimulus and
c) determining the response to said immune stimulus by said cells,

wherein an impaired response compared to the response in the absence of said test molecule is an indication that said molecule under test is a agonist of CD36 and/or CD51 and/or a thrombospondin receptor.

78. A method as claimed in claim 77 wherein said thrombospondin receptor is not CD47.

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79. A method as claimed in claim 77 or claim 78 wherein said response is maturation of said antigen-presenting cell.

5 80. A method as claimed in any one of claims 77 to 79 wherein said antigen-presenting cell of the immune system is selected from a dendritic cell, a macrophage, a B-lymphocyte or a monocyte.

10 81. A method as claimed in any of claims 77 to 80 which includes the features of any of claims 16 to 18 or 21 to 32.

15 82. A method of treating mammalian antigen-presenting cells in vitro to induce immune tolerance therein which comprises exposing said cells to an agonist of the cell surface receptor for thrombospondin and/or an agonist for the cell surface receptors CD36 and/or CD51.

20 83. A method as claimed in claim 82 wherein said antigen-presenting cell is human or mouse.

25 84. A method as claimed in claim 82 or 83 wherein said antigen presenting cell is selected from a dendritic cell, a macrophage, a B-lymphocyte or a monocyte.

30 85. A method as claimed in any one of claims 82 to 84 wherein said agonist is a molecule identified as such by the method of any one of claims 77 to 81.

35 86. A method as claimed in any one of claims 82 to 85 which includes the features of any one of claims 4 to 12.

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87. A method as claimed in any one of claims 82 to 85 wherein said antigen-presenting cells are exposed to an antibody to an epitope of a thrombospondin receptor.

5

88. A method as claimed in claim 87 wherein said agonist is any one of the antibodies listed in Appendix 3.

10

89. A method as claimed in claim 87 or claim 88, which includes the features of any of claims 9 to 12.

15

90. An antigen-presenting cell preparation obtainable by the method of any one of claims 82 to 89 for use as a medicament.

20

91. As antigen-presenting cell preparation obtainable by the method of any one of claims 82 to 89 for use in inducing peripheral immune tolerance in a human.

25

92. A method of identifying a molecule which is an agonist of a β -integrin associated with the cell surface receptor CD51 as expressed on antigen-presenting cells of the mammalian immune system which method comprises:

30

a) exposing mammalian antigen-presenting cells to the molecule to be tested,

b) exposing said cells to an immune stimulus and

c) determining the response to said immune stimulus by said cells,

35

wherein an impaired response compared to the response in the absence of said test molecule is an indication that said molecule is an agonist of a β -integrin associated with the cell surface receptor CD51.

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93. A method as claimed in claim 92 which includes the features of claim 78 or claim 79

5 94. A method as claimed in claim 92 to 93 which further includes the features of any one of claims 16 to 25 or 29 to 32.

10 95. A method as claimed in any one of claims 92 to 94 wherein said β -integrin is $\beta 3$ or $\beta 5$.

96. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of a β -integrin associated with the cell surface receptor CD51 as expressed on
15 mammalian antigen-presenting cells and a pharmacologically acceptable carrier or diluent.

20 97. A pharmaceutical composition as claimed in claim 96 wherein the β -integrin is $\beta 3$ or $\beta 5$.

98. A method of inducing a state of immune tolerance in antigen-presenting cells of the mammalian immune system which comprises exposing said cells ex-vivo to an agonist of a β -integrin.

25 99. A method as claimed in claim 97 wherein said β -integrin is $\beta 3$ or $\beta 5$.

30 100. A method as claimed in claim 97 to 98 wherein said antigen presenting cells are human.

101. A method as claimed in claim 97 or 99 wherein said cells are exposed to an antigenic material.

35 102. A preparation of cells obtainable by the method as claimed in any one of claims 97 to 100 for

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use as a medicament.

103. A preparation of cells obtainable by the
method as claimed in any one of claims 97 to 100 for
5 use in inducing immune-tolerance in a human.

104. A pharmaceutical composition suitable for
inducing peripheral immune tolerance in a mammal which
comprises an agonist of a thrombospondin receptor and
10 a pharmacologically acceptable carrier or diluent.

105. A composition as claimed in claim 104
wherein said thrombospondin receptor is not CD47.

15 106. A preparation of apoptotic cells for use in
inducing peripheral immune tolerance in a mammal.

107. A preparation as claimed in claim 106
wherein said mammal is a human.

20 108. A preparation comprising a negatively
charged phospholipid for use in inducing peripheral
immune tolerance in a mammal.

25 109. A preparation as claimed in claim 108
wherein said mammal is a human.

110. A preparation as claimed in claim 108 or 109
which comprises liposomes including a negatively
30 charged phospholipid.

111. A preparation as claimed any one of claims
108 to 110 wherein said negatively charged
phospholipid is phosphatidylserine.

35 112. A method of inducing a state of immune
tolerance in antigen-presenting cells of the mammalian

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immune system which comprises exposing said cells ex-vivo to a composition or preparation as claimed in any one of claims 104 to 111.

5 113. A method as claimed in claim 112 wherein said cells are exposed to an antigenic material.

114. A preparation of antigen-presenting cells obtainable by the method of claims 112 or 113.

10

115. A method of treating a human to induce peripheral immune tolerance therein comprising administering to said human a substance selected from the group consisting of: an agonist of CD36, an
15 agonist of CD51, an agonist of a thrombospondin receptor, an agonist of a β -integrin and a preparation of cells of any of claims 13, 14, 90, 91, 102 or 114.

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CLAIMS:

1. A method of treating mammalian dendritic cells in vitro to induce immune tolerance therein which comprises exposing said cells to an agonist of the cell surface receptors CD36 and/or CD51 as expressed on mammalian dendritic cells; wherein said agonist is selected from: an antibody with an affinity for an epitope of CD36, an antibody with an affinity for an epitope of CD51, the Plasmodium falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1, thrombospondin, apoptotic cells or a negatively charged phospholipid.

2. A method as claimed in claim 1 wherein said dendritic cells are human or mouse.

3. A method as claimed in claim 1 or 2 which comprises exposing said dendritic cells to two or more of the agonists of claim 1.

4. A method as claimed in any one of claims 1 to 3 wherein the pf-EMP-1 active binding domain comprises the amino acid sequence shown in Figure 2.

5. A method as claimed in any one of claims 1 to 4 wherein said cells are exposed to an antigenic material selected from: an auto-antigen associated with a particular auto-immune disease, an allo-antigen, a xeno-antigen or a therapeutic substance which is likely to induce an unwanted therapeutic response.

6. A method as claimed in claim 5 wherein the cells so produced are subsequently matured by exposure to an immune stimulus.

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7. A method as claimed in any of claims 1 to 6 wherein said dendritic cells are prepared from human peripheral blood and or derived from CD34+ stem cells or monocytes.

5

8. A dendritic cell preparation obtainable by the method of any of claims 1 to 7 for use as a medicament.

10

9. A dendritic cell preparation obtainable by the method of any of claims 1 to 7 for use in inducing peripheral immune tolerance in a human.

15

10. A method of identifying a molecule which is an agonist of cell surface receptor CD36 and/or CD51 as expressed by mammalian dendritic cells which method comprises:

a) exposing immature mammalian dendritic cells to the molecule to be tested,

20

b) exposing said immature dendritic cells to an immune stimulus and

c) determining the degree of maturation manifested by said dendritic cells,

25

wherein impaired maturation in response to the immune stimulus is an indication that said molecule under test is a potential CD36 and/or CD51 agonist; wherein if said test molecule is found to be a potential agonist of CD36 and/or CD51 the method further comprises the step of exposing said molecule to a purified sample of CD36 and/or CD51 and detecting any direct binding between said molecule and CD36and/or CD51.

30

11. A method as claimed in claim 10 wherein said dendritic cells are human cells or mouse cells.

35

12. A method as claimed in claim 10 or 11

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wherein maturation of said dendritic cells is determined by examining the antigen-presenting ability of said cells.

5 13. A method as claimed in any of claims 10 to 12 wherein maturation of said dendritic cells is determined by examining said cells for expression of at least one cell surface antigen whose level of expression is enhanced in response to an immune
10 stimulus.

 14. A method as claimed in claim 13 wherein maturation of said dendritic cells is determined by measuring the level of expression of one or more of
15 the following panel of antigens:
HLA DR, CD54, CD40, CD83 and CD86.

 15. A method as claimed in claim 14 wherein said cells are also examined for expression of CD80.
20

 16. A method as claimed in any one of claims 13 to 15 wherein the level of expression of said antigens is detected using a labelled antibody.

25 17. A method as claimed in 10 or 11 wherein maturation of said dendritic cells is determined by measuring said cells' ability to induce T-cell proliferation.

30 18. The method of claim 10 or 11 wherein maturation of said dendritic cells is determined by quantifying the level of cytokines secreted from said cells.

35 19. The method of claim 18 wherein the level of secretion of TNF α , IL12P70 and IL10 is measured.

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20. A method as claimed in any one of claims 10 to 19 wherein said immune stimulus is lipopolysaccharide, TNF α , CD40L or monocyte conditioned medium (MCM).

5

21. A method as claimed in any one of claims 10 to 20 wherein said purified CD36 or CD51 is immobilised to a solid surface.

10

22. A method as claimed in any one of claims 10 to 21 wherein said molecule is labelled with a detectable label.

15

23. A method as claimed in any one of claims 10 to 22 which further comprises the step of exposing said molecule to a purified sample of $\alpha_v\beta_3$ or $\alpha_v\beta_5$ and detecting any direct binding between said molecule and said $\alpha_v\beta_3$ or $\alpha_v\beta_5$.

20

24. A method as claimed in any one of claims 10 to 23 which further comprises the step of exposing said molecule to a purified sample of thrombospondin and detecting any direct binding between said molecule and thrombospondin.

25

25. A method as claimed in claim 23 or claim 24 wherein said molecule is labelled with a detectable label.

30

26. A method as claimed in any of claims 23 to 25 wherein said $\alpha_v\beta_3$, $\alpha_v\beta_5$ or thrombospondin is immobilised to a solid surface.

35

27. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of the cell surface receptor CD36 as expressed on mammalian dendritic cells and a

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pharmacologically acceptable carrier or diluent.

28. A composition as claimed in claim 27 which is suitable for inducing peripheral immune tolerance in a human wherein said CD36 agonist is selected from: an antibody with an affinity for an epitope of CD36, the Plasmodium falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1 for CD36 and/or thrombospondin, thrombospondin, apoptotic cells or a negatively-charged phospholipid.

29. A composition as claimed in claim 28 wherein the pf-EMP-1 binding domain comprises the amino acid sequence as shown in Figure 2.

30. Use of an agonist of the cell surface receptor CD36 as expressed on mammalian dendritic cells in the manufacture of a medicament to induce a state of immune tolerance in a human.

31. The use as claimed in claim 30 wherein said CD36 agonist is selected from: an antibody with an affinity for an epitope of CD36, the Plasmodium falciparum protein pf-EMP-1, a protein comprising the active binding domain of pf-EMP-1 for CD36 or thrombospondin, thrombospondin, apoptotic cells or a negatively charged phospholipid.

32. The use as claimed in claim 31 wherein the pf-EMP-1 active binding domain comprises the amino acid sequence as shown in Figure 2.

33. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of the cell surface receptor CD51 as expressed by mammalian dendritic cells and a pharmacologically acceptable carrier or diluent.

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34. A composition as claimed in claim 33
suitable for inducing immune tolerance in a human
wherein said CD51 agonist is selected from: an
antibody with an affinity for an epitope of CD51,
5 thrombospondin, apoptotic cells or a negatively
charged phospholipid.

35. A composition as claimed in claim 33 which
comprises the Plasmodium falciparum protein pf-EMP-1
10 or a protein comprising an active binding domain
thereof and thrombospondin.

36. A composition as claimed in claim 35 wherein
said active binding domain of pf-EMP-1 comprises the
15 amino acid sequence shown in Figure 2.

37. Use of an agonist of the cell surface
receptor CD51 as expressed on mammalian dendritic
cells in the manufacture of a medicament for inducing
20 a state of immune tolerance in a human.

38. Use of an agonist as claimed in claim 37
wherein said CD 51 agonist is selected from: an
antibody with an affinity for an epitope of CD51,
25 thrombospondin, apoptotic cells or a negatively
charged phospholipid.

39. The use of an agonist as claimed in claim 37
wherein said medicament comprises, in combination, the
30 Plasmodium falciparum protein pf-EMP-1 or a protein
comprising an active binding domain thereof and
thrombospondin.

40. A method of identifying a molecule which is
35 an agonist of cell surface receptors CD36 and/or CD51
and/or a receptor for thrombospondin as expressed on
antigen-presenting cells of the mammalian immune

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system which method comprises:

a) exposing mammalian antigen-presenting cells to the molecule to be tested,

b) exposing said cells to an immune stimulus and

5 c) determining the response to said immune stimulus by said cells,

wherein an impaired response compared to the response in the absence of said test molecule is an indication that said molecule under test is a
10 potential agonist of CD36 and/or CD51 and/or a thrombospondin receptor and wherein if said test molecule is found to be a potential agonist of CD36 and/or CD51 and/or a thrombospondin receptor, the method further comprises the step of exposing said
15 molecule to a purified sample of CD36 and/or CD51 and/or thrombospondin and detecting any direct binding between said molecule and CD36 and/or CD51 and/or thrombospondin.

20 41. A method as claimed in claim 40 wherein said thrombospondin receptor is not CD47.

42. A method as claimed in claim 40 or claim 41 wherein said response is maturation of said
25 antigen-presenting cell.

43. A method as claimed in any one of claims 40 to 42 wherein said antigen-presenting cell of the immune system is selected from a dendritic cell, a
30 macrophage, a B-lymphocyte or a monocyte.

44. A method of treating mammalian antigen-presenting cells in vitro to induce immune tolerance therein which comprises exposing said cells
35 to an agonist of the cell surface receptor for thrombospondin and/or an agonist for the cell surface receptors CD36 and/or CD51.

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45. A method as claimed in claim 44 wherein said mammalian antigen presenting cell is a dendritic cell.

5 46. A method as claimed in claim 44 or claim 45 wherein said antigen-presenting cell is human or mouse.

10 47. A method as claimed in claim 44 or 45 wherein said antigen presenting cell is selected from a macrophage, a B-lymphocyte or a monocyte.

15 48. A method as claimed in any one of claims 44 to 47 wherein said antigen-presenting cells are exposed to an antibody to an epitope of a thrombospondin receptor.

20 49. An antigen-presenting cell preparation obtainable by the method of any one of claims 44 to 48 for use as a medicament.

25 50. As antigen-presenting cell preparation obtainable by the method of any one of claims 44 to 48 for use in inducing peripheral immune tolerance in a human.

30 51. A method of identifying a molecule which is an agonist of a β -integrin associated with the cell surface receptor CD51 as expressed on antigen-presenting cells of the mammalian immune system which method comprises:

a) exposing mammalian antigen-presenting cells to the molecule to be tested,

b) exposing said cells to an immune stimulus and

35 c) determining the response to said immune stimulus by said cells,

wherein an impaired response compared to the response in the absence of said test molecule is an

- 9 -

indication that said molecule is an agonist of a β -integrin associated with the cell surface receptor CD51.

5 52. A method as claimed in 51 wherein said β -integrin is β_3 or β_5 .

10 53. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of a β -integrin associated with the cell surface receptor CD51 as expressed on mammalian antigen-presenting cells and a pharmacologically acceptable carrier or diluent.

15 54. A pharmaceutical composition as claimed in claim 53 wherein the β -integrin is β_3 or β_5 .

20 55. A method of inducing a state of immune tolerance in antigen-presenting cells of the mammalian immune system which comprises exposing said cells ex-vivo to an agonist of a β -integrin.

25 56. A method as claimed in claim 55 wherein said β -integrin is β_3 or β_5 .

30 57. A method as claimed in claim 55 or 56 wherein said antigen presenting cells are human.

35 58. A method as claimed in any one of claims 55 to 57 wherein said cells are exposed to an antigenic material selected from an auto-antigen associated with a particular auto-immune disease, an allo-antigen, a xeno-antigen or a therapeutic substance which is likely to induce an unwanted immune response.

 59. A preparation of cells obtainable by the method as claimed in any one of claims 55 to 58 for

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use as a medicament.

5 60. A preparation of cells obtainable by the method as claimed in any one of claims 54 to 57 for use in inducing immune-tolerance in a human.

10 61. A pharmaceutical composition suitable for inducing peripheral immune tolerance in a mammal which comprises an agonist of a thrombospondin receptor and a pharmacologically acceptable carrier or diluent.

62. A composition as claimed in claim 61 wherein said thrombospondin receptor is not CD47.

15 63. A preparation of apoptotic cells for use in inducing peripheral immune tolerance in a mammal.

64. A preparation as claimed in claim 63 wherein said mammal is a human.

20 65. Use of a preparation comprising a negatively charged phospholipid in the manufacture of a medicament for use in inducing peripheral immune tolerance in a mammal.

25 66. The use as claimed in claim 65 wherein said mammal is a human.

30 67. The use as claimed in claim 65 or 66 wherein said medicament comprises liposomes including a negatively charged phospholipid.

35 68. The use as claimed any one of claims 65 to 67 wherein said negatively charged phospholipid is phosphatidylserine.

69. A method of inducing a state of immune

- 11 -

tolerance in antigen-presenting cells of the mammalian immune system which comprises exposing said cells ex-vivo to a composition or preparation as defined in any one of claims 61 to 68.

5

70. A method as claimed in claim 69 wherein said cells are exposed to an antigenic material selected from: an auto-antigen associated with a particular auto-immune disease, an allo-antigen, a xeno-antigen or a therapeutic substance which is likely to induce an unwanted immune response.

10

71. A preparation of antigen-presenting cells obtainable by the method of claims 69 or 70.

15

72. A method of treating a human to induce peripheral immune tolerance therein comprising administering to said human a substance selected from the group consisting of: an agonist of CD36, an agonist of CD51, an agonist of a thrombospondin receptor, an agonist of a β -integrin and a preparation of cells of any of claims 8, 9, 49, 50, 59, 60 or 71.

20

73. A method of identifying a molecule capable of preventing the adherence of red blood cells infected with a malarial parasite to human dendritic cells which comprises:

25

a) exposing immature human dendritic cells to the Plasmodium falciparum protein pf-EMP-1 or an active binding domain thereof in the presence or absence of the molecule to be tested,

30

b) exposing said immature dendritic cells to an immune stimulus and

c) determining the degree of maturation manifested by said dendritic cells,

35

wherein any maturation of said dendritic cells in the presence of the test molecule over and above that

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manifested in the absence of said molecule is an indication that said molecule is capable of preventing adherence of red blood cells infected with a malarial parasite to human dendritic cells.

5

74. A method as claimed in claim 73 wherein maturation of said dendritic cells is determined by examining the antigen-presenting ability of said cells.

10

75. A method as claimed in claim 73 or claim 74 wherein maturation of said dendritic cells is determined by examining said cells for expression of at least one cell surface antigen whose expression level is enhanced in response to an immune stimulus.

15

76. A method as claimed in claim 75 wherein maturation of said dendritic cells is determined by measuring the level of expression of two or more of the following panel of antigens:
HLA DR, CD54, CD40, CD83 and CD86.

20

77. A method as claimed in claim 76 wherein said cells are also examined for expression of CD80.

25

78. A method as claimed in any one of claims 75 to 77 wherein the level of expression of said antigen is detected using a labelled antibody.

30

79. A method as claimed in claim 73 wherein maturation is determined by measuring said cells' ability to induce T-cell proliferation.

35

80. A method as claimed in claim 73 wherein maturation of said dendritic cells is determined by quantifying the level of cytokines secreted from said cells.

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81. A method as claimed in claim 80 wherein the level of secretion of TNF α , IL12P70 and IL10 is measured.

5 82. A method as claimed in any one of claims 73 to 81 wherein said immune stimulus is lipopolysaccharide, TNF α , CD40L or monocyte conditioned medium (MCM).

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10/019580

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<120> INDUCTION OF IMMUNE TOLERANCE

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<170> PatentIn Ver. 2.0

<210> 1

<211> 444

<212> PRT

<213> Plasmodium falciparum

<400> 1

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Leu	Cys	Ile	Phe	Glu	Lys	Met	Lys	Gly	His	Lys	Lys	Val	Lys	Lys	Gln
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Arg Ala Gly Gly Arg Lys Thr Leu Arg Ala Asp Ala Ser Gln Gly His 405 410 415		
Tyr Asn Gly Lys Ala Asn Glu Ser Val Leu Lys Asp Val Cys Asp Ile 420 425 430		
Thr Asn Gln Tyr Ser Asn Ala Ile Gly Asp Ser Lys Asp 435 440 445		

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(74) Agent: **BOULT WADE TENNANT**; Verulam Gardens,
70 Gray's Inn Road, London WC1X 8BT (GB).

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(71) Applicant (for all designated States except US): **ISIS IN-
NOVATION LTD.** [GB/GB]; Ewert House, Ewert Place,
Summertown, Oxford OX2 7BZ (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROBERTS, David,**
John [GB/GB]; National Blood Service Oxford Centre,
John Radcliffe Hospital, Headington, Oxford OX3 9DU
(GB). **URBAN, Britta, Christina** [DE/GB]; Institute of
Molecular Medicine, John Radcliffe Hospital, Headington,
Oxford OX3 9DS (GB).

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For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: TREATMENT OF DENDRITIC CELLS FOR INDUCTION OF IMMUNE TOLERANCE

(57) Abstract: Methods and compositions for the induction of immune tolerance in mammalian antigen presenting cells such as dendritic cells, macrophages, monocytes and B-lymphocytes are described. Such methods and compositions involve the use of agonists of the cell surface receptors CD36, CD51, thrombospondin receptors and/or the β -integrins which when exposed to an antigen-presenting cell such as a dendritic cell are able to inhibit maturation therein. Thus, the cells' ability to promote an immune response is inhibited. Tolerance to a specific antigen can be induced in antigen-presenting cells by exposure to one or more of the aforesaid agonists and the antigen. Thus, cell preparations can be prepared for administration to humans where tolerance to a specific antigen or antigens needs to be induced, for example in the case of allograft or xenograft transplants or in autoimmune disease.

WO 01/02005 A3

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 00/02546

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N5/08 G01N33/50 A61K39/395 A61K39/005 A61K38/17
 A61K35/14 A61K39/00 A61P37/00 A61P33/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, MEDLINE, LIFESCIENCES, CHEM ABS Data, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 06848 A (BLOOD RES CENTER) 15 April 1993 (1993-04-15) the whole document	33-35, 38-41, 54-62, 75,76
X	MCCORMICK C J ET AL: "Intercellular adhesion molecule-1 and CD36 synergize to mediate adherence of Plasmodium falciparum-infected erythrocytes to cultured human microvascular endothelial cells." JOURNAL OF CLINICAL INVESTIGATION, vol. 100, no. 10, 15 November 1997 (1997-11-15), pages 2521-2529, XP000971964 the whole document -/--	54-62, 75,76

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

11 January 2001

Date of mailing of the international search report

25/01/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Teyssier, B

INTERNATIONAL SEARCH REPORT

Inten Application No

PCT/GB 00/02546

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 05191 A (UAB RESEARCH FOUNDATION) 23 February 1995 (1995-02-23) the whole document	33,34, 38-40, 43,44, 46, 49-51, 53,75, 104-107
X	WO 96 33736 A (AFFYMAX TECH NV ;BARUCH DROR I (US); HOWARD RUSSELL J (US); PASLOS) 31 October 1996 (1996-10-31) the whole document	33,34, 36, 38-40, 42,43, 46,47, 49-51, 53,75, 104-107
X	WO 90 15609 A (MED TAL INC) 27 December 1990 (1990-12-27) the whole document	108-111
A	ALBERT M L ET AL: "Immature dendritic cells phagocytose apoptotic cells via alpha V beta 5 and CD36, and cross-present antigens to cytotoxic T lymphocytes" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 188, no. 7, 5 October 1998 (1998-10-05), pages 1359-1368, XP000906793	
P,X	URBAN B C ET AL: "Modulation of dendritic cell maturation and function." TISSUE ANTIGENS, vol. 55, no. Supplement 1, 2000, page 61 XP000971966 7th Workshop and Conference on Human Leucocyte Differentiation Antigens; Harrogate, England; 20-24 June 2000 abstract I. 19	1-115
P,X	URBAN B C ET AL: "Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells." NATURE, vol. 400, no. 6739, 1 July 1999 (1999-07-01), pages 73-77, XP002156922 the whole document	1-115

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-53, 77-115

Methods of treating antigen presenting cells, including dendritic cells, to induce immune tolerance; the resulting antigen presenting cells preparations and their use as a medicament.

Methods of identifying agonists of CD36 or CD51; pharmaceutical compositions containing CD36 or CD51 agonists and their use as a medicament.

Methods of identifying agonists of a beta-integrin associated with CD51. Pharmaceutical composition containing a beta-integrin agonist and a CD51 agonist. Use of a beta-integrin agonist to induce tolerance in antigen presenting cells; tolerised cell composition.

Pharmaceutical composition containing a thrombospondin agonist. Use of a thrombospondin agonist to induce tolerance in antigen presenting cells; tolerised cell composition.

Pharmaceutical composition containing a negatively charged phospholipid. Use of a negatively charged phospholipid to induce tolerance in antigen presenting cells; tolerised cell composition.

2. Claims: 54-76

Methods of identifying a molecule capable of preventing the adherence of red blood cells infected with a malarial parasite to human dendritic cells; said molecule and pharmaceutical compositions comprising it.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

1/ Lack of clarity

Claims 1-3, 33, 37-39, 43, 46-50, 53, 73-76, 82-85, 87 and 115 relate to compounds, to pharmaceutical compositions comprising compounds or to methods using compounds in which said compounds are defined solely by reference to desirable characteristics or properties, namely the agonism of the cells surface receptors CD36 and/or CD51 or the prevention of the adherence of red blood cells infected with a malarial parasite to human dendritic cells. The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, disclosed and supported, namely, antibodies to CD36 and/or CD51 (as applicable), Pf-EMP-1, thrombospondin, fragments and derivatives of said antibodies and proteins, and negatively charged phospholipids, as mentioned in claims 4, 6-8, 34-36, 40-42, 44, 45, 51, 52 and 88.

2/ Inconsistencies in claims 9, 35-37 and 102-103

Claim 9 relates to "a method as claimed in any one of claims 40 to 47" while said claims 40-47 relate to compounds and compositions thereof; claim 9 is therefore inconsistent. In view of the context of these claims, this International Search Authority assumed that claim 9 refers to claims 1-8.

Claims 35-36 and 37 relate to a composition as claimed in claim 20 and 19, respectively, while said claims 20 and 19 relate to a method of identifying ligands; claims 35-37 are therefore inconsistent. In view of the context of these claims, this International Search Authority assumed that claims 35-36 refer to claim 34 and that claim 37 refers to claim 33.

Claims 102-103 relate to "a preparation of cells obtainable by the the method as claimed in any one of claims 97 to 100" while claim 97 relates to a pharmaceutical composition of a beta-integrin. Claims 98-100 do relate to a method suitable to obtain a composition of claims 102-103. Consequently, the part of claims 102-103 which relate to claim 97 was disregarded.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. Application No

PCT/GB 00/02546

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9306848	A	15-04-1993	AU 2795892 A	03-05-1993
			AU 2797892 A	03-05-1993
			AU 2861292 A	03-05-1993
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			US 5135922 A	04-08-1992
			US 5262406 A	16-11-1993

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